U.S. Dept. of agriculture

JOURNAL OF AGRICULTURAL RESEARCH

VOLUME 34
JANUARY 1 – JUNE 15, 1927

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE
WITH THE COOPERATION OF THE ASSOCIATION
OF LAND-GRANT COLLEGES

UNITED STATES
GOVERNMENT PRINTING OFFICE
WASHINGTON
1927

LIBRARY

JOINT COMMITTEE ON POLICY AND MANUSCRIPTS

FOR THE UNITED STATES DEPARTMENT OF AGRICULTURE

E. W. ALLEN, CHAIRMAN
Chief, Office of Experiment Stations

C. L. SHEAR
Senior Pathologist in Charge, Plant Disease Survey and Pathological Collections

A. C. BAKER
Senior Entomologist in Charge, Tropical and Subtropical Plant Insect Investigations

FOR THE ASSOCIATION OF LAND-GRANT COLLEGES

R. W. THATCHER
Director, New York Experiment Stations

S. B. HASKELL
Director, Massachusetts Experiment Station

M. J. FUNCHESS
Director, Alabama Experiment Station

EDITORIAL SUPERVISION

M. C. MERRILL
Editorial Chief of Publications, United States Department of Agriculture

All correspondence regarding articles from State experiment stations should be addressed to R. W. Thatcher, Agricultural Experiment Station, Geneva, N. Y.

Published on the first and fifteenth of each month. This volume will consist of twelve numbers and the Contents and Index.

Subscription price: Domestic, $4.00 a year (two volumes)
Single numbers, 20 cents
Foreign, $5.25 a year (two volumes)
Single numbers, 25 cents

If separates are desired in quantity, they should be ordered at the time the manuscript is sent to the printer, and they will be supplied practically at cost. Single copies of separates may be obtained free of charge from the Office of Information, United States Department of Agriculture, until the supply is exhausted.

Address all correspondence regarding subscriptions and purchase of numbers and separates to the Superintendent of Documents, Government Printing Office, Washington, D. C.

630.61
U58j v.34

282307
D.C.

DARTMOUTH COLLEGE LIBRARY

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 34 WASHINGTON, D. C., JANUARY 1–JUNE 15, 1927

CONTENTS

	Page
Development of Flowers and Seed in the Sugar Beet. ERNST ARTSCHWAGER (1 plate; 15 text figures)	1
Some Variations of the Heat Method for Sterilizing Milking Machines. L. H. BURGWALD	27
A Biochemical Study of the False-Blossom Disease of the Cranberry. C. P. SPAETH and H. R. KRAYBILL	35
The Nitrogen Metabolism of Steers on Rations Containing Alfalfa as the Sole Source of the Nitrogen. HARRY W. TITUS (1 text figure)	49
Injury to Growing Crops Caused by the Application of Arsenical Compounds to the Soil. H. E. MORRIS and D. B. SWINGLE (6 text figures)	59
Studies of Commercial Sauerkraut with Special Reference to Changes in the Bacterial Flora During Fermentation at Low Temperatures. LILLIAN A. PRIEM, W. H. PETERSON, and E. B. FRED (4 text figures)	79
Effect of Phosphorus on the Composition of the Tomato Plant. JOHN H. MACGILLIVRAY (6 text figures)	97
Critical Tests of Tetrachlorethylene as an Anthelmintic for Foxes. KARL B. HANSON	129
Effect of Carbon Dioxide upon the Germination of Chlamydospores of *Ustilago zeae* (Beckm.) Ung. G. A. PLATZ, L. W. DURRELL, and MARY F. HOWE (3 text figures)	137
Notes on the Life History and Control of the Strawberry Leaf Roller. E. W. DUNNAM (3 text figures)	149
A Study of Dark, Hard Kernel and Protein Content of Hard Red Spring Wheat. C. E. MANGELS (6 text figures)	157
The Computed as Compared with the Directly Observed Fasting Katabolism of Cattle as a Measure of the Maintenance Requirement of Energy. E. B. FORBES, MAX KRISS, and WINFRED W. BRAMAN	167
The Inheritance of Length of Style in Buckwheat. R. J. GARBER and K. S. QUISENBERRY	181
Self-Fertilization in Buckwheat. R. J. GARBER and K. S. QUISENBERRY (1 text figure)	185
Effect of Smut (*Ustilago zeae*) on the Sugar Content of Cornstalks. ANNIE MAY HURD-KARRER and HEINRICH HASSELBRING	191
Studies on Tomato Wilt Caused by *Fusarium lycopersici* Sacc. RICHARD P. WHITE (7 plates; 9 text figures)	197
A Comparison of Some Physical and Chemical Tests for Determining the Quality of Gluten in Wheat and Flour. D. A. COLEMAN, H. B. DIXON, and H. C. FELLOWS	241
The Life History, Habits, and Economic Importance of Some Mononchs. GERALD THORNE (6 text figures)	265
Two Water Molds Causing Tomato Rootlet Injury. CHARLES DRECHSLER (2 text figures)	287
The Value of Beef Protein as a Supplement to the Proteins in Certain Vegetable Products. RALPH HOAGLAND and GEORGE G. SNIDER	297
The Nitrogen Compounds of the Rice Kernel as Compared with Those of Other Cereals. S. L. JODIDI	309
Peridermium kurilense Diet. on *Pinus pumila* Pall., and *Peridermium indicum* N. Sp. on *Pinus excelsa* Wall. REGINALD H. COLLEY and MINNIE W. TAYLOR (1 text figure)	327
New Species of Chalcid Flies Parasitic on the Gipsy-Moth Parasite, *Apanteles melanoscelus* (Ratzeburg). C. F. W. MUESEBECK	331

Page

Effect of Soil Microorganisms on Paraffin Used as a Coating to Decrease the Injurious Action of Lead Arsenate on Plant Roots. WALTER E. FLEMING 335

The Determination of Organic Matter in Soils by Means of Hydrogen Peroxide. W. O. ROBINSON 339

Diplodia Ear-Rot Disease of Corn. E. E. CLAYTON 357

Measures of Persistency of Lactation. W. L. GAINES. (1 text figures) 373

The Vitamin C Content of Fresh and Canned Pear. VERRAL C. CRAVEN and MARTHA M. KRAMER (3 text figures) 385

Sterility and Fertility in the Strawberry. GEORGE M. DARROW (14 text figures) 393

A Study of the Factors Determining Quality in Sweet Corn. C. W. CULPEPPER and C. A. MAGOON (6 text figures) 413

Relation of Soil Temperature and Soil Moisture to the Infection of Sweet Potatoes by the Stem-Rot Organisms. L. L. HARTER and W. A. WHITNEY (2 text figures) 435

A Transit Disease of Snap Beans Caused by *Pythium aphanidermatum*. L. L. HARTER and W. A. WHITNEY (1 plate) 443

A Technic for Use with Homopterous Vectors of Plant Disease, with Special Reference to the Sugar-Beet Leaf Hopper, *Eutettix tenellus* (Baker). WALTER CARTER (1 text figure) 449

New Species and New Forms of Ichneumonidae Parasitic upon the Gipsy-Moth Parasite, *Apanteles melanoscelus* (Ratzeburg). R. A. CUSHMAN 453

Dispersion of the Angoumois Grain Moth to Wheat Fields. PEREZ SIMMONS and GEORGE W. ELLINGTON 459

Two Hymenopterous Parasites of American Jointworms. W. J. PHILLIPS and F. W. POOS (5 text figures) 473

A Canker of Apple and Pear Trees Caused by *Glutinium macrosporum*, N. Sp. S. M. ZELLER (10 text figures) 489

The Results of Inoculating *Pinus strobus* with the Sporidia of *Cronartium ribicola*. HARLAN H. YORK, WALTER H. SNELL, and ANNIE RATHBUN-GRAVATT (1 plate; 3 text figures) 497

A Morphologic and Biometric Comparison of *Cronartium ribicola* and *Cronartium occidentale* in the Aecial Stage. REGINALD H. COLLEY, CARL HARTLEY, and MINNIE W. TAYLOR (5 text figures) 511

Burrknot Formations in Relation to the Vascular System of the Apple Stem. CHARLES F. SWINGLE (5 plates; 4 text figures) 533

Four New Chalcidoid Parasites of the Pine Tip Moth, *Rhyacionia frustrana* (Comstock). A. B. GAHAN 545

The Critical Temperature of the Chicken. H. H. MITCHELL and W. T. HAINES (1 text figure) 549

Hereditary and Environmental Factors that Produce Mottling in Soy Beans. F. V. OWEN (1 plate; 4 text figures) 559

Pigeon Pea Anthracnose. C. M. TUCKER (3 text figures) 589

Preliminary Studies on the Relation of Fire Injury to Bark-Beetle Attack in Western Yellow Pine. J. M. MILLER and J. E. PATTERSON (7 text figures) 597

The Parasite of the Pine Tip Moth, *Rhyacionia frustrana* (Comstock). R. A. CUSHMAN (1 text figure) 615

Adaptation of the Bacterial Action of Chloroform to the Preparation of Bacterins. HUBERT BUNYEA (2 text figures) 623

The Natural Replacement of Blight-Killed Chestnut in the Hardwood Forests of the Northeast. C. F. KORSTIAN and PAUL W. STICKEL (9 text figures) 631

Isolation and Identification of Some Organic Nitrogenous Compounds Occurring in Etiolated Corn Seedlings. S. L. JODIDI 649

The Effect of Applications of Cyanamid on the Nitrate Content of Field Soils. F. E. ALLISON 657

Effect of Pumping from Deep Wells on the Ground-Water Table. WALTER W. WEIR (10 text figures) 663

Reciprocal Effects from Grafting. FRED W. HOFMANN 673

Influence of Form and Proportion of Lime Used and of Method of Mixing on the Resulting Bordeaux Mixture. E. B. HOLLAND, C. O. DUNBAR, and G. M. GILLIGAN 677

The Morphological Differentiation of the Pistillate Flowers of the Pecan. D. V. SHUHART (6 plates; 5 text figures) 687

Page

A Cytological Study of Orange Leaf Rust, *Puccinia triticina* Physiologic Form 11, on Malakoff Wheat. RUTH F. ALLEN (7 plates) 697

The Swine Herdbook as a Source of Data for the Investigation of the Sex Ratio and Frequency of Sex Combinations in Pig Litters. HUGH C. MCPHEE (1 text figure) 715

Relation of the Size of Oil Drops to Toxicity of Petroleum-Oil Emulsions to Aphids. EDWARD L GRIFFIN, CHARLES H. RICHARDSON, and ROBERT C. BURDETTE (2 text figures) 727

Three New Hymenopterous Parasites of the Pine Tip Moth, *Rhyacionia frustrana* (Comstock). R. A. CUSHMAN 739

Eurytoma parva (Girault) Phillips and Its Biology as a Parasite of the Wheat Jointworm, *Harmolita tritici* (Fitch). W. J. PHILLIPS (2 text figures) 743

The Rate of Passage of Food Through the Digestive Tract of the Hen. M. HELEN KEITH, L. E. CARD, and H. H. MITCHELL 759

Some Factors Other than Bacteria that Influence the Body of Artificial Buttermilk. GEORGES KNAYSI (1 text figure) 771

Net-Energy Values of Corn Silage, Soy-Bean Hay, Alfalfa Hay, and Oats. E. B. FORBES, WINFRED W. BRAMAN, MAX KRISS, J. AUGUST FRIES, C. D. JEFFRIES, R. W. SWIFT, ROWLAND B. FRENCH, and J. V. MAUCHER, Jr. 785

Run-Off from Small Agricultural Areas. C. E. RAMSER (15 text figures) 797

Strains of Kernel Smuts of Sorghum, *Sphacelotheca sorghi* and *S. cruenta*. W. H. TISDALE, L. E. MELCHERS, and H. J. CLEMMER (4 text figures) 825

Anthelmintic Properties of Santonin. JACOB E. SHILLINGER 839

The Oviposition Rate of the Grape Leaf Hoppers. CHESTER I. BLISS (1 text figure) 847

An Undescribed White Fly Attacking Citrus in Porto Rico. H. L. DOZIER (3 text figures) 853

The Yellow Rust of Raspberry Caused by *Phragmidium imitans*. S. M. ZELLER (4 text figures) 857

A Comparison of the Direct Measurement of the Heat Production of Cattle with the Computation of the Heat Production by the Respiratory-Quotient Method. E. B. FORBES, MAX KRISS, WINFRED W. BRAMAN, and R. B. FRENCH 865

Sabal causiarum (Cook) Beccari: A new Host of the Coconut Bud-Rot Fungus. C. M. TUCKER. (4 text figures) 879

The Discoloration of Canned Cranberries. FRED W. MORSE 889

Mottle Necrosis of Sweet Pototoes. L. L. HARTER and W. A. WHITNEY (6 text figures) 893

The Comparative Susceptibility of Sweet-Potato Varieties to Stem Rot. L. L. HARTER and W. A. WHITNEY (1 text figure) 915

Inheritance of Rate of Shedding in a Cotton Hybrid. THOMAS H. KEARNEY and ROBERT H. PEEBLES 921

The Basal Metabolism of Mature Chickens and the Net-Energy Value of Corn. H. H. MITCHELL and W. T. HAINES 927

The Effect of Age, Sex, and Castration on the Basal Heat Production of Chickens. H. H. MITCHELL, L. E. CARD, and W. T. HAINES 945

The Inheritance of Ovate and Related Shapes of Tomato Fruits. E. W. LINDSTROM (8 text figures) 961

The Gossypol Content and Chemical Composition of Cottonseeds during Certain Periods of Development. WILLIS D. GALLUP 987

Variants in *Ustilago nuda* and Certain Host Relationships. W. H. TISDALE and MARION A. GRIFFITHS 993

Development of the Female Gametophyte and Caryopsis of *Poapratensis* and *Poa compressa*. ALICE M. ANDERSEN (9 plates; 3 text figures) 1001

Life Histories and Heterothallism of the Red Bread-Mold Fungi of the *Monilia sitophila* Group. C. L. SHEAR and B. O. DODGE (4 plates) 1019

A Study of the Toxicity of Acid Lead Arsenate on the Japanese Beetle (*Popillia japonica* Newm.) E. R. VAN LEEUWEN 1043

The Development of the Pecan Nut (*Hicoria pecan*) from Flower to Maturity. J. G. WOODROOF and NAOMI CHAPMAN WOODROOF (10 text figures) 1049

Sulphuric Acid as a Weed Spray. ALFRED ASLANDER (5 text figures) 1065

The Water Requirement of Plants at Akron, Colo. H. L. SHANTZ and LYDIA N. PIEMEISEL (16 text figures) 1093

ERRATA AND AUTHORS' EMENDATIONS

Page 143, Table 4, last column, "5.83" should be "5.30."
Page 177, fourth line from bottom, "1.313" and "1.537" should be "1,313" and "1,537."
Page 288, lines 3 and 4, should be interchanged.
Page 445, third line from bottom, footnote numeral 6 should be inside parenthesis, thus: "*P. myriotylum* Drechsler, ined.[6])"
Page 451, in heading, "Organism" should be "Virus."
Page 564, first line of legend of Figure 1, insert after "branch" "(the lowest only)."
Page 657, line 12, "coordinated" should be "correlated."
Page 729, Table 1, heading of the fifth column should be "Density, 20° C."
Page 781, lines 9 and 23, "Hcl" should be "HCl."
Page 798, Table 1, seventh column, unit under "Time of concentration," "Minimum" should be "Minutes."
Page 801, near middle of page, formula should be "$Q=(3.83-0.07L)\ Lh^{(1.32+0.01L)}$," that is, the quantity in the second parenthesis is an exponent.
Page 806, Table 5, last column, references to Figures 9, 10, 11, and 12 should be to Figures 11, 12, 13, and 14, respectively.
Page 913, Figure 6 is reversed
Page 930, the table and the paragraph printed in small type belong to the footnote at bottom of page.
Page 1026, line 24 (end of par. 3), "pl. 2" should be "pl. 4."
Page 1027, eighteenth line from bottom, "Plate 2" should be "Plate 4."
Page 1031, line 17 "(pl. 3, A, B, C)," should be "(pl. 1, C–F)."
Page 1039, line 8, "readily" should be "really."
Page 1041, citation (25), delete "[Original not seen. Abstract in Rev. Appl. Mycol. 4: 707–708. 1925]."
Page 1043, line 8, "20 per cent" should be "30 per cent."
Page 1054, line 6, "indelible" should be "inedible."
Page 1094, fifth line from bottom, "national" should be "natural."
Page 1173, Table 33, "Tumbleweed," "Pigweed," "Russian thistle," "Lamb's quarters," and "Polygonum" should have a separate heading, "Weeds."
Page 1173, "Table 33, first column, under "Wheat: C. I. 4131," "S. P. I. 3715" should be "S. P. I. 37159."

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 34 WASHINGTON, D. C., JANUARY 1, 1927 No. 1

DEVELOPMENT OF FLOWERS AND SEED IN THE SUGAR BEET [1]

By ERNST ARTSCHWAGER

Associate Pathologist, Office of Sugar Plants, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In comparison with its anatomy, which has been the subject of extensive research, the flower development of the sugar beet has received scant attention. Practically all previous work was unmeditated, and we look in vain for a consecutive account of the development of the flower and fruit. Very recently, however, after the work recorded in this paper had been completed and submitted for publication, there appeared a paper by Dudok van Heel (*2*) [2] in which the cytology and embryogeny of the sugar beet had been worked out in much detail with all important features illustrated by means of semi-diagrammatic drawings. This paper, however, complete in all other respects, does not take account of the general development of the flower and the relationship of the leaf and floral traces.

MATERIALS AND METHODS

The material used in the present study was grown at Fort Collins and Fort Lewis, Colo. The different strains had been selected and grown by W. W. Tracy for a number of years and possessed a great uniformity in foliage and root characters. Since the strains were grown separately in well-isolated localities they seemed suited for this work, especially since pure lines are unknown and commercial stock is a hopeless mixture. Material was fixed in Carnoy's fluid—three parts of absolute alcohol and one part of acetic acid. Other material was fixed in Allen's modification of Bouin's solution which is suitable for counting chromosomes in certain stages. The material was embedded in paraffin, cut from 5 to 15 μ thick, and stained with Haidenhain's iron-alum haematoxylin.

DEVELOPMENT OF THE SEED BUSH

The cultivated beet is a biennial and develops its seed during the second year. When planted out the second season the mother beets first produce a rosette of leaves like those of the first year, but after about six weeks of growth the newly formed leaves become progressively smaller and finally the apical portion begins to elongate,

[1] Received for publication May 18, 1926; issued January, 1927.
[2] Reference is made by number (italic) to "Literature cited," p. 25.

thus initiating the development of the floral axis. The latter grows with great rapidity, but the leaves which develop on this shoot are much smaller than the rosette leaves and more widely spaced. In the axils of the leaves shoots arise which also grow rapidly and give rise to branches of the third order. The mature inflorescence or "seed bush" is composed of large, paniculate, more or less open, spikes bearing the flowers and later the seed.

The sessile flowers are usually in clusters of two or three (fig. 1, C) which are attached to the inflorescence axis or secondary branches of the latter. The small, greenish flowers (fig. 1, A) are perfect, the calyx is 5-parted and incurved, adhering to the base of the ovary and becoming hard in the fruit. There are five stamens inserted at the base of the calyx lobes. The stamens are introrse, bilocular, and open by longitudinal fissures. The 3-carpellary ovary is sunk in a fleshy disk inclosing a campylotropous ovule which is attached laterally to the ovary wall by a short funiculus. The style is very short, terminating in a 3-lobed stigma which persists in the fruit. The latter is an aggregate, formed by the cohesion of two or more flowers grown together at their bases and forming a hard and irregular dry body, the so-called "seed ball."

An illustrated account of the development of the flower of *Beta maritima* is given by Payer (*7, p. 310–311, pl. 66*), and with it the description of the sugar-beet flower, as given below, tallies. The rudiments of the young flowers appear close behind the growing point of branches of the inflorescence axis. Each rudiment is borne in the axil of a large bract and is accompanied by two lateral bracts each of which has in its axil a flower. The flower primodium forms a small protuberance on which are soon differentiated five narrow ridges, the beginnings of the sepals. Next there appears within this circle a whorl of papillae, the stamen rudiments each opposite a calyx lobe. Finally three more ridges develop at first separately but later joining to inclose a more or less compressed roundish cavity in which a single ovule develops.

Once initiated, the primordia of the different floral parts develop very rapidly. The young stamens become stalked early and attain in cross section the characteristic quadrilocular form. The anther is at first a homogeneous mass of tissue covered by an epidermis. As soon as it becomes faintly 4-lobed in cross section the cells next to the epidermis begin to elongate radially and divide periclinally, forming two layers of narrow cells which by their size and staining reaction appear distinctly set off from the central tissue which forms the archesporium. These two layers of cells together with the epidermis constitute the anther wall. The cells of the middle layer, in their subsequent development, enlarge rapidly and finally develop thickening bands. This constitutes the endothecium and forms a continuous mantle except in the region where the anther later dehisces. The inner layer is in places more than one cell wide. The cells remain small and as the anther enlarges they become stretched tangentially, but they do not become obliterated before the endothecium is fully developed and the pollen practically mature.

The ovule arises near the base of the carpels. It is seen first as a slight protuberance which enlarges to form the nucellus. In its subsequent development the young ovule becomes distinct from the

surface of the carpel by a stalklike base or funiculus. Very early there appears at the base of the nucellus an annular outgrowth followed soon by a second one of a similar nature. The first outgrowth

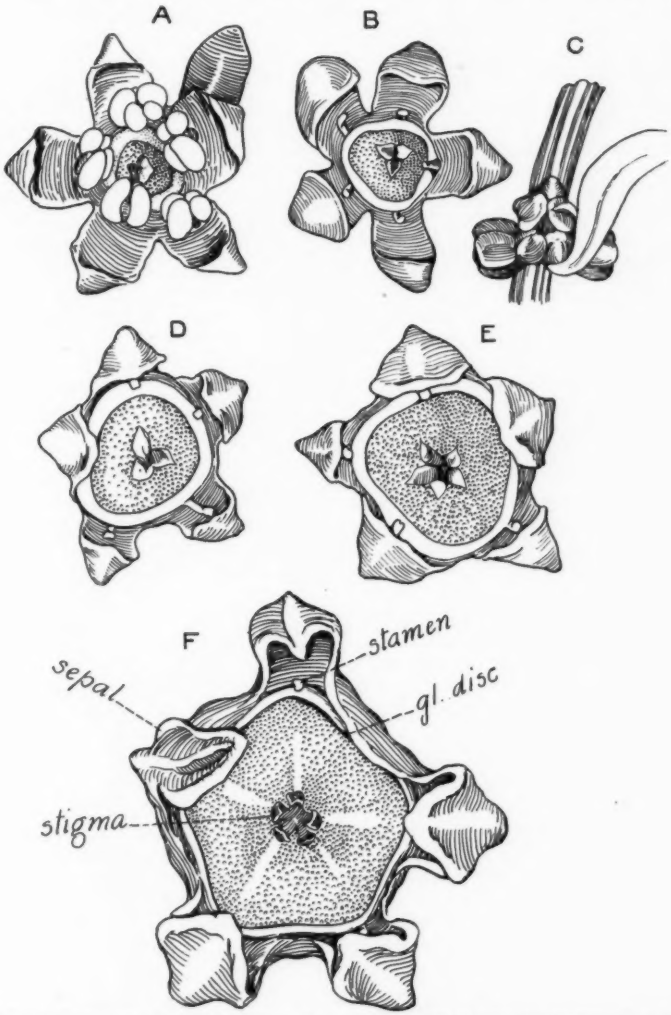


FIG. 1.—A, single mature flower viewed from above; B, flower with stamens already dropped; C, cluster of flowers attached to branch of inflorescence axis and subtended by a bract; D, flower after fertilization; E, flower with young embryo; F, flower with mature embryo. All × 10.25

develops into the inner, the second into the outer integument. The young ovule is straight, but with the appearance of the integuments it curves and becomes campylotropous. Finally, the ovule twists about its own funiculus and comes to lie horizontally in the cavity of

the ovary. This change in position as pointed out by Meunier (*6*) is necessitated by the form of the ovary cavity and made possible by the structure of the funiculus.

ANATOMY OF THE FLORAL AXIS

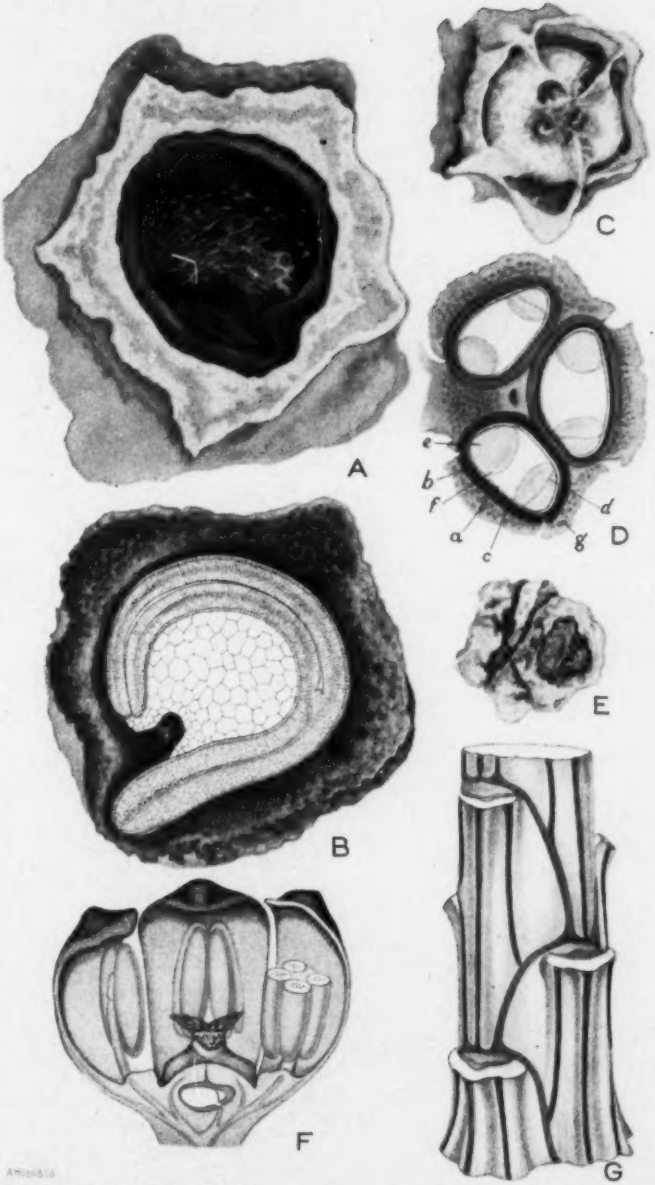
A transverse section through the young floral axis shows a circle of vascular bundles limited on the inside by a well-developed pith, on the outside by the epidermis and cortex (fig. 2, B). The pith is homogeneous, but the cortex possesses ridges of collenchymatous tissue near the periphery. The epidermis is one cell thick and abuts directly on the collenchyma or cortex.

The vascular tissue follows naturally a longitudinal course in the axis and its arrangement is closely related to the phyllotaxis, which is, however, often subject to variation. The normal arrangement is alternate with a divergence of two-fifths; that is, leaf number six stands directly over leaf number one after two turns of the spiral. Frequently the lower leaves of the inflorescence appear in pairs whereby a pseudo-opposite arrangement is effected. In traversing the stem vertically the bundles remain at approximately the same distance from the center except in the node, where certain bundles approach and leave the periphery alternately and acquire a somewhat undulate course.

The vascular bundles as seen in cross section are of different magnitude. Occupying the corners of the irregular polygon (fig. 2, B) are five large cauline bundles alternating with an equal number of strands, usually somewhat smaller in size, which represent the median traces of the first five leaves. Flanking the sides of the cauline bundles are small strands of vascular tissue which represent the lateral traces. Plate 1, G, and Figure 3 show the course and the origin of the different traces and their relation to each other and to the stem. A study of the petiole of a very young leaf shows that there are three foliar traces, a large central one and two smaller ones occupying the flanks of a flattened semicircle. As a rule, however, there are many more bundles even in the youngest petioles, so that it is very difficult to follow the course of these various strands and to establish their origin. But neglecting the variations that are commonly met with, the derivation of the traces accords with the following plan: Each new median trace is derived from a left cauline bundle (viewed from the stem apex) a little distance above the node, it ascends without fusion or forking for five internodes and passes into the leaf without branching. The left lateral trace is derived from the cauline bundle next it, passes through three internodes, and usually branches several times before entering the leaf. The right

EXPLANATORY LEGEND FOR PLATE 1

A.—Mature seed in its natural position in cavity of ovary
B.—Seed cut open to show the embryo
C.—Part of seed ball with "lid" still closing the cavity of the ovary in which the seed is contained. The remains of the sepals are seen folding over the lid
D.—Section through seed ball containing three seeds. The curved embryos are seen in cross section. *a*, Lid closing the cavity of the ovary; *b*, sclerenchymatous tissue; *c*, seed testa; *d*, cotyledons of embryo; *e*, radicle of embryo; *f*, perisperm; *g*, loose spongy tissue
E.—Seed ball containing three seeds
F.—Young flower cut open to show its different parts and the course and origin of the vascular traces
G.—Portion of inflorescence axis five internodes in length. The leaf-traces are drawn in color to show more clearly their derivation and course. The cauline bundles are colored green, the median traces red, and the lateral bundles blue


A
B
C
D
e
b
f
a
c
g
d
E
F
G

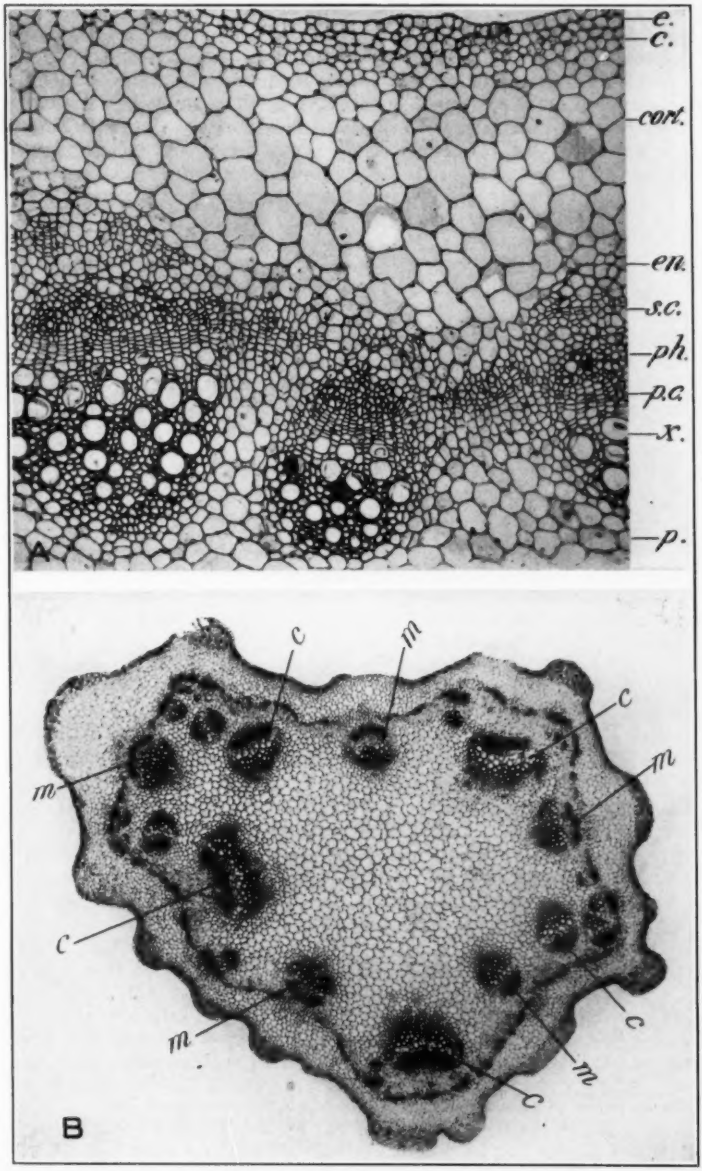


FIG. 2.—A, partial cross section of floral axis: *e*, epidermis; *c*, collenchyma; *cort*, cortex; *en*, endodermis; *s. c.*, secondary cambium; *ph*, phloem; *p. c.*, primary cambium; *x*, xylem; *p*, pith. × 144. B, cross section through young floral axis; *c*, cauline bundles; *m*, median trace. × 24.5

lateral trace is also derived from its adjacent cauline bundle. It passes through only two internodes, but otherwise conforms to the left lateral trace. The length of the lateral trace is, however, subject to variation since it often remains a part of the cauline bundles for a longer or shorter portion of their ascent.

In each node, then, the cauline bundles flanking the oldest median trace undergo the following changes: A little distance below, or

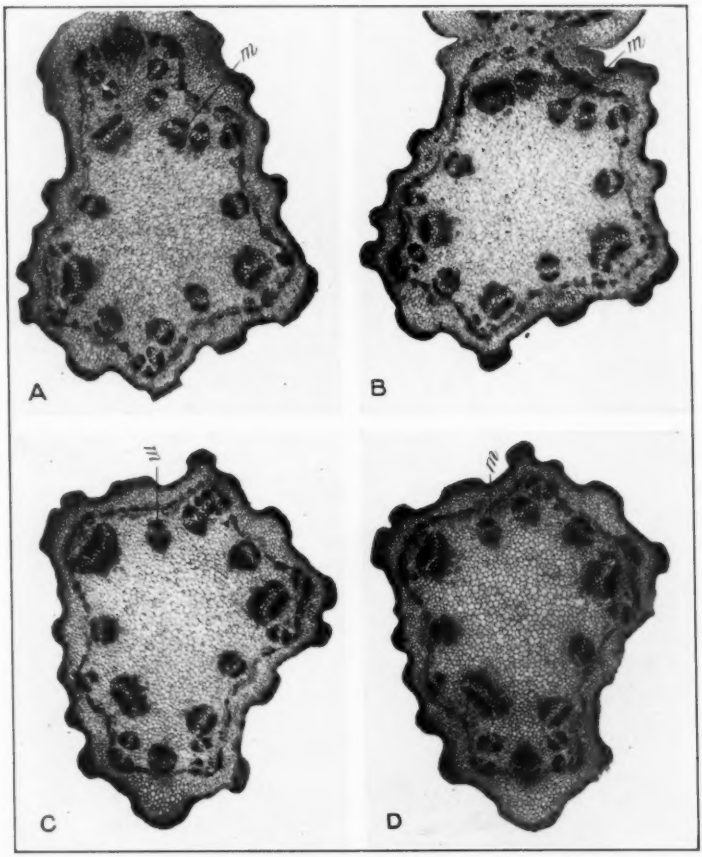


FIG. 3.—A, Cross section through floral axis just below a node; B, the same but taken at the insertion point of the leaf; C, the same but taken just below the node of the next higher leaf; D, the same but taken a little higher up the stem. All × 14.25
The new median trace is designated with *m*, note its position in the different cuts

sometimes above the insertion of the leaf each cauline bundle gives off a small branch which becomes a new lateral trace; a little higher up they approach each other and may even temporarily fuse. Upon separation, the left cauline bundle branches once more and the new strand which is segregated becomes a new median trace, as shown in

Plate 1. The vascular supply of the axillary members is also derived from the cauline bundles a short distance below the node.

Fron (*1*) describes and pictures the origin and course of the vascular bundles in the floral axis of *Beta cicla* and states that the course of the bundles in *Beta vulgaris* is similar. According to Fron, the median trace splits at a distance of three internodes below its point of entrance, and the two resulting bundles pass into the pith for a short distance in their downward course and after traversing two or three more internodes attach themselves to their adjacent cauline bundles just below the node at which the latter enter the stem.

Although the vascular skeleton of the floral axis of the sugar beet shows a great deal of variation, the differences are not fundamental and never of a type which would conform to Fron's account.

The traces to the various floral organs are joined to the peduncle by a short receptacular stele in which the vascular tissue forms a somewhat irregular central core instead of the usual ring of separate bundles. With the broadening of the receptacle five distinct groups of vascular tissue separate off and diverge to occupy a position in the peripheral region of the cortex, while the remaining tissue of the core unites into a single bundle which becomes continuous with the vascular supply of the ovule. (Fig. 4, A, B.) Simultaneous and progressive changes are now seen in all parts. The periphery of the receptacle becomes distinctly 5-lobed and into these lobes vascular strands pass which have separated from the five cortical bundles (fig. 4, C); these bundles later branch again to form the lateral traces of the calyx lobes. From the central portion of the receptacle the ovary is now formed. The surrounding tissue constitutes the three fused carpels to the side of one of which a single ovule is attached by a short funiculus. Fundamentally, each carpel is supplied with three vascular strands—a central bundle and two marginal ones. In the flower of the sugar beet the marginal traces have been suppressed so that each carpel is supplied with only a single trace. These traces are derived from the cortical bundles in such a manner that two of them constitute direct branches of two cortical bundles lying opposite them, while the third carpellary trace is formed as a fusion bundle of two vascular strands derived from the adjacent cortical bundles. Occasionally these two bundles remain separate so that a cross section through the upper part of the pistil shows four bundles instead of three. Ultimately, these bundles fuse or one of them becomes reduced and ends blindly in the tissue of the carpel. What remains of the five cortical bundles after the sepals and carpels have been supplied, passes out into the stamens, each of which has a single trace (fig. 4, D). The course of the vascular supply in the flower and the mode of origin and course of the traces supplying the different floral organs is illustrated in the semidiagrammatic sketch (fig. 4, A to D). Plate 1, F, shows a still unopened flower with its vascular supply in median longitudinal section.

SECONDARY GROWTH IN THE FLORAL AXIS

After the formation of the primary bundles in which a normal cambium functions for a certain length of time, an interfascicular cambium differentiates. The segments of this cambium are formed in such a way that one flank of a segment becomes continuous with

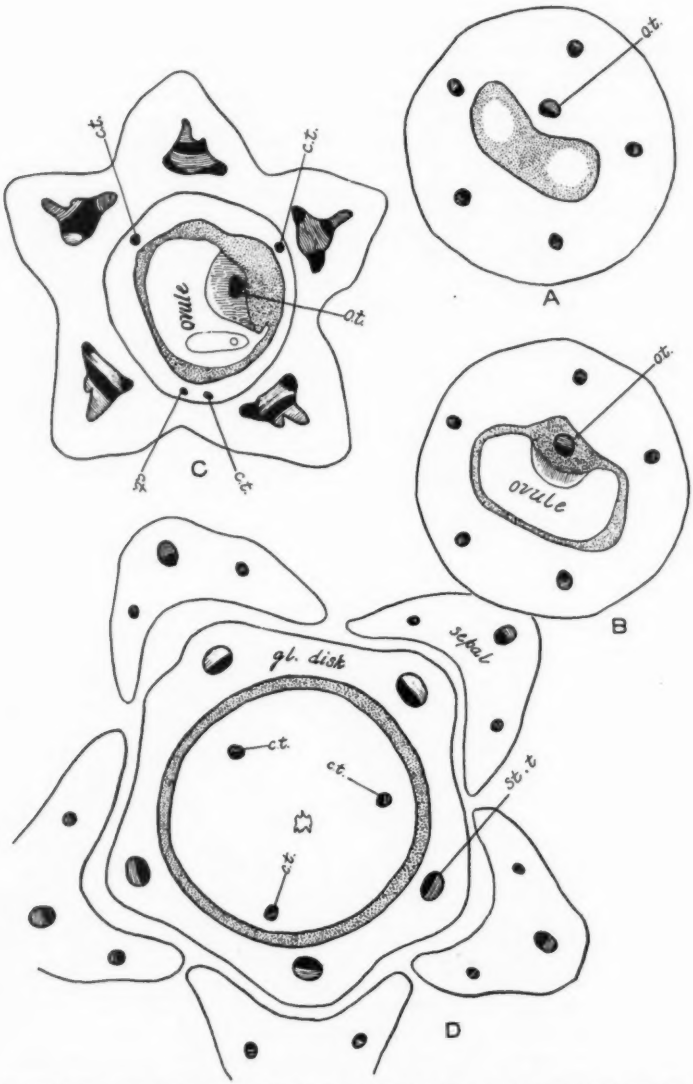


FIG. 4.—Diagrammatic drawings of a series of sections through a flower to illustrate the origin and course of the vascular supply: A, section through base of receptacle. There are present five peripheral bundles and a sixth one *o. t.* in close proximity to the base of the ovary cavity; B, section taken at a slightly higher level. Bundle *o. t.* is now seen in the cavity of the ovary; it forms the vascular supply of the single ovule; C, section taken at a level where the sepals are differentiating. The peripheral bundles are branching. Four strands designated *c. t.* have separated to form the vascular supply of the three carpels; D, section through the upper part of the flower. The sepals are now distinct. The filaments of the stamens are still a part of the glandular disk region. Each stamen is supplied with but a single trace

the normal cambium of the bundle while the other end arches around the phloem of another bundle, as shown in Figure 2, A. This method of unilateral connection was first noted by Hérail (*4*) and later substantiated by Fron (*1*) for other Chenopodiaceae.

As the activity of the normal cambium ceases, a new cambium arises in the pericycle so that at this stage the cambium ring is in part normal, in part pericyclic. Later it becomes entirely pericyclic.

Cell division in the new cambium is at first centripetal whereby xylem and parenchyma are formed. After a limited period of centripetal growth the cambium forms phloem and parenchyma centrifugally. The segments of cambium later mature completely into vascular tissue and parenchyma, whereby the ring of cambium is again broken. Before this occurs new segments of cambium are differentiated in the parenchyma on the outer phase of the phloem, and these new segments unite with the older cambium on either side. The new cambium undergoes the usual reciprocal divisions producing xylem and phloem. This process is repeated as long as active growth in the stem continues.

MICROSPOROGENESIS

The cells of the archesporial tissue of the young anther develop rapidly to the mother-cell stage. At the beginning of meiosis these cells show a net work of dense granular cytoplasm; the nuclei are large and contain a single nucleolus lying in a more or less central position. The chromatin is in the nature of fine granules distributed over a delicate linen net. Fine threads sometimes extend to the nucleolus which otherwise appears as if suspended in a hyaline vacuole. The chromatin granules are of uniform size and tend to aggregate along the nuclear membrane. Gradually the linen net becomes more permanent, the number of threads increases, and the threads themselves become thicker and the granules larger (fig. 5, A). The nucleolus shows frequently an appearance of budding. The protuberances separate off and diffuse out into the reticulum, becoming identical with the chromatin there present.

Without showing distinct pairing, the chromatin threads now contract toward one side of the nucleus. During this period of maximum contraction (fig. 5, B) the synaptic mass is very dense and the association of the chromatin threads becomes very intimate. The passing out of synizesis is first manifested by loops of free spireme being thrown into the nuclear cavity (fig. 5, C). These continue to spread and soon the entire chromatin cord is distributed through the nuclear cavity forming the well-known hollow spireme (fig. 5, D). The larger portion of the spireme is arranged along the nuclear membrane, but it also traverses the cavity in various directions. The appearance of the nucleus after synizesis differs from the preceding condition since the chromatic elements pass into synapsis as distinct bodies and emerge as a uniform filament.

The synaptic stages are somewhat prolonged and the loosening and the formation of the spireme also take place gradually so that the persistence of the "Klumpen" stage, as also shown by Dudok van Heel (*2*), is only apparent, and what appears to be a *Klumpen* when viewed under high power is only an intermediate stage; that is, the

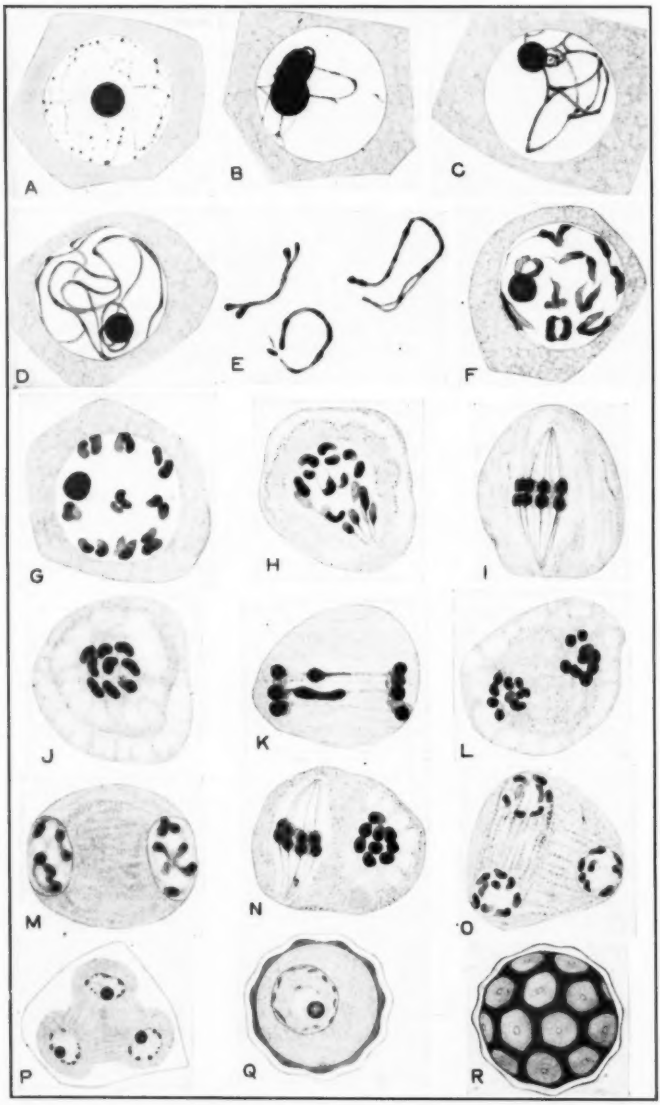


FIG. 5.—Meiosis in microspore development: A, early prophase; B, synizesis; C, postsynizesis; D, hollow spireme; E, segmentation of spireme to form the chromosomes; F, early diakinesis; G, late diakinesis; H, multipolar spindle; I, metaphase; J, polar view of metaphase; K, anaphasic separation; L, late anaphase; M, telophase; N, homotypic spindles; O, homotypic telophase; P, tetrad formation; Q, young pollen grain; R, mature pollen grain, surface view

Klumpen in the process of being formed or in the initial stage of its loosening. However, the subsequent stages as well as the homotypic division are passed through very rapidly.

Without going into second contraction the previously paired spireme threads now segment into pieces of various lengths which are twisted hither and thither in the nuclear cavity. The segments commonly have the form of a U (fig. 5, E), but they may be of various shape. The chromosomes or gemini now undergo the usual thickening and shortening and eventually attain a compact form. The various pairs are at first distributed throughout the nuclear cavity (fig. 5, F), but gradually they become limited to the nuclear membrane (fig. 5, G). The nucleolus at this stage is slightly reduced in size and occupies an eccentric position which makes the counting of the chromosomes more difficult. The appearance of the chromosomes in diakinesis does not deviate from the normal condition. There are commonly nine pairs present with no unpaired univalents, but owing to the smallness of the chromosomes it is not always possible to determine the latter point with certainty.

The arrangement of the chromosomes in diakinesis is quickly followed by the disappearance of the nuclear membrane and the nucleolus. A multipolar spindle is differentiated (fig. 5, H), but as the chromosomes approach the equator the spindle becomes distinctly bipolar (fig. 5, I). The disappearance of the nuclear membrane and the formation of the multipolar spindle are so quickly accomplished that one rarely sees these stages in a preparation. Cross sections through an anther often show the chromosomes of one locule of the theca in diakinesis, while in the other locule they are already arranged on the equator. Since in the sugar beet any given meiotic stage occurs simultaneously throughout a locule, it becomes necessary to study cross sections in order to determine the proper sequence of the different stages and their relative duration; this is especially necessary when the stages are passed through rapidly.

At the equatorial plate the chromosome are seen as thick, more or less roundish, lumps which in polar view appear distributed over a more or less circular space (fig. 5, J). The two members of each bivalent now separate in metakinesis, one going to each pole. On their way to the pole certain chromosomes may show lagging (fig. 5, K), but on the whole the anaphasic separation is quite normal. After a very brief interkinesis (fig. 5, M), the chromosomes of each daughter nucleus pass on to the homotypic spindle (fig. 5, N). The two spindles may lie parallel or at right angles to each other. On the spindle the chromosome halves separate and thus the longitudinal split is completed.

The nuclear plate in the heterotypic anaphase contains nine chromosomes. These are at this stage almost spherical and as a rule lie well apart. Winge (*10; p. 178*) and Dukok van Heel (*2*) also found nine chromosomes to be characteristic for Beta and for other members of the Chenopodiaceae. Matthysen's (*5*) finding of eight chromosomes in the cultivated garden beet is at variance with these figures and in the light of the present account is open to question. However, the writer also was able to count but eight chromosomes

in some of his sugar-beet material, but such small irregularities should be of no significance.

After the second division (fig. 5, O, P), each of the young microspores becomes invested by a wall which at first is thin and delicate. As the spore increases in size, the wall becomes differentiated into two layers (fig. 5, Q). The outer layer is thick and sculptured and contains numerous thin spots for the exit of the pollen tube (fig. 5, R). The microspores become free from one another at maturity, forming a powdery mass which, when the anther dehisces, is distributed by wind and insects.

The tapetal layer in the developing anther is separated from the primary sporogenous tissue after the three layers of the anther wall have become distinct. Although showing the same staining reaction, the young tapetal cells can be distinguished from the sporogenous tissue not only by their peripheral location but also by the shape and size of the individual cells. When the sporogenous cells enter the mother-cell stage, the cells of the tapetum begin to enlarge radially, but axially their length remains about the same. In stained sections they appear darker than the sporogenous cells and this feature persists until they disintegrate. At about the time the sporogenous cells enter synapsis the tapetal cells become binucleated. By this time the cells have increased their radial diameter to such an extent that the anticlinal walls are more than double the length of the periclinal ones (fig. 6, A, and fig. 7, A). However, not all of the cells are narrow; some of them enlarge tangentially as well as radially and appear almost square in cross section, while still others are polygonal or otherwise irregular. The nuclei of the tapetal cells are conspicuous by the large quantity of dark-staining granules. There is commonly only one nucleolus, but occasionally some of the chromatin granules are so large as to simulate secondary nucleoli. The cytoplasm of the cells is also very dense and takes a darker stain than the cytoplasm of the sporogenous cells. At the time of the appearance of the tetrads the tapetum cells show signs of degeneration (fig. 7, B). They gradually lose their content which is being absorbed by the developing pollen. The nuclei, however, retain their granular appearance for a long time, but finally they too become disorganized. Their walls remain intact until the pollen is mature, but they collapse prior to the dehiscence of the anther and the shedding of the pollen (fig. 6, B).

It is evident from the foregoing description that the nuclear phenomena in microsporogenesis of the sugar beet conform generally to the process in other plants (*8*). The early prophase stages appear complex and have been described differently by Matthysen (*5*), who studied meiosis in the garden beet. But since details in cell division may be studied profitably only in organisms possessing large nuclei, little is gained by attempting it with structures so small as the chromosomes of the sugar beet. However, since genetical researches carried on in connection with breeding problems require for a background a knowledge of chromosome behavior in postsynizesis rather than an exact interpretation of the early prophase stages, the object of this investigation has been realized.

MACROSPOROGENESIS

The archesporium develops from a single hypodermal cell of the nucellus. The epidermal cells divide several times tangentially whereby the sporogenous cell of the megasporocyte becomes embedded in

FIG. 6.—A, partial cross section through tapetum at a stage when the sporogenous tissue is in late prophase; B, section through mature anther. The pollen is fully formed while the cells of the tapetum have practically degenerated. × 820

nucellar tissue (fig. 8, A). At the close of the first meiotic division in the megasporocyte a wall is formed between the two nuclei, but the two daughter cells divide rapidly again to form the four megaspores (fig. 8, B). The outer three megaspores degenerate while the inner

one forms the embryo sac. The young embryo sac is straight, but soon the nucellar cells at the chalazal end begin to degenerate and as the ovule continues to curve disintegration of the nucellar tissue also proceeds, until a more or less horseshoe-shaped cavity results. The curvature of the embryo sac is hastened by cell division in the concave part of the nucellus (fig. 9, A), and through these divisions a tissue is produced which later becomes filled with starch and which forms the perisperm of the mature seed. The cavity of the embryo sac is lined with broken cell remains. At the basal end of the mature sac an accumulation of protoplasm is commonly found with numerous embedded nuclei. The nucleus of the functional megaspore initiates the usual series of three equational divisions with the resulting nuclei lying free in the embryo sac. The egg and the two synergids

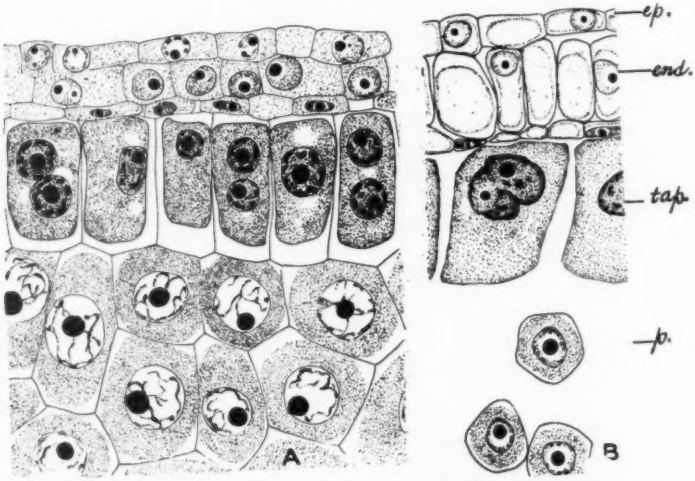


FIG. 7.—A, cross section through wall layer, tapetum, and peripheral sporogenous tissue of a young anther; B, an older stage. Note that the second wall layer has greatly enlarged while the cells of the third layer have almost completely degenerated. The tapetal cells are at this stage already in the incipient stages of degeneration

are located in the micropylar end of the sac. The two synergids partly surround the larger egg which bears its nucleus in the end toward the center of the sac (fig. 10, A). The two polar nuclei fuse some time before fertilization. The resulting fusion nucleus is very large with a well-marked nucleolus and a dense reticulum. The antipodal cells which are found in the chalazal end of the sac are well developed. They begin to degenerate before the embryo sac is mature, but often only after the egg is fertilized.

FERTILIZATION AND EMBRYOLOGY

The mature beet flowers begin to open in the morning; the sepals expand horizontally, leaving the stamens exposed and in an upright position. If the day is warm and sunny the anthers dehisce in the forenoon and the pollen is carried by wind and insects to the receptive stigmas of older flowers. Shaw (*9*) considered thrips to be the most

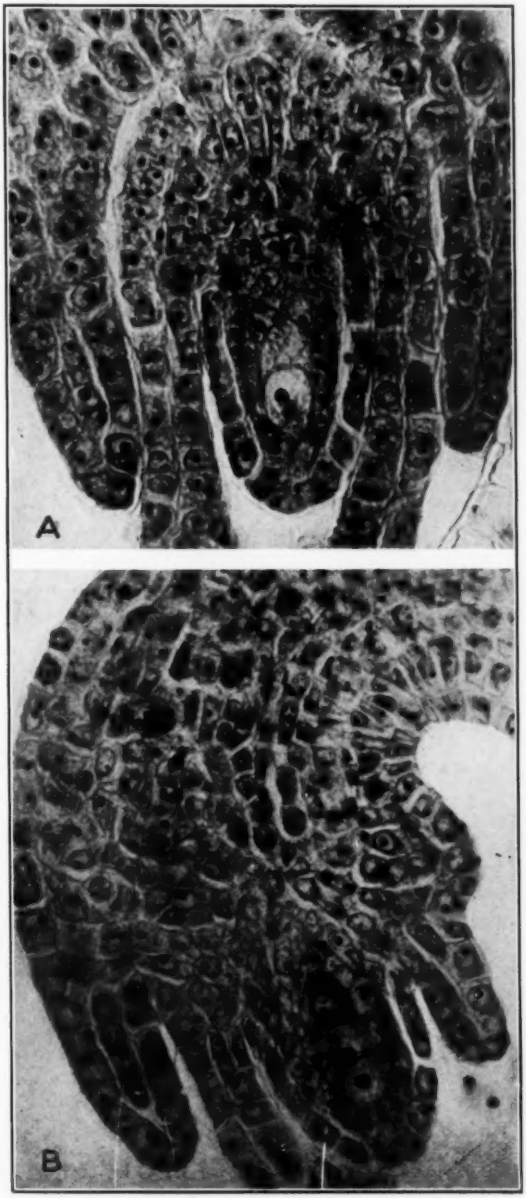


FIG. 8.—A, longitudinal section through young ovule with megaspore mother cell, × 700; B, longitudinal section through a megaspore tetrad. The innermost cell becomes the embryo sac, × 700

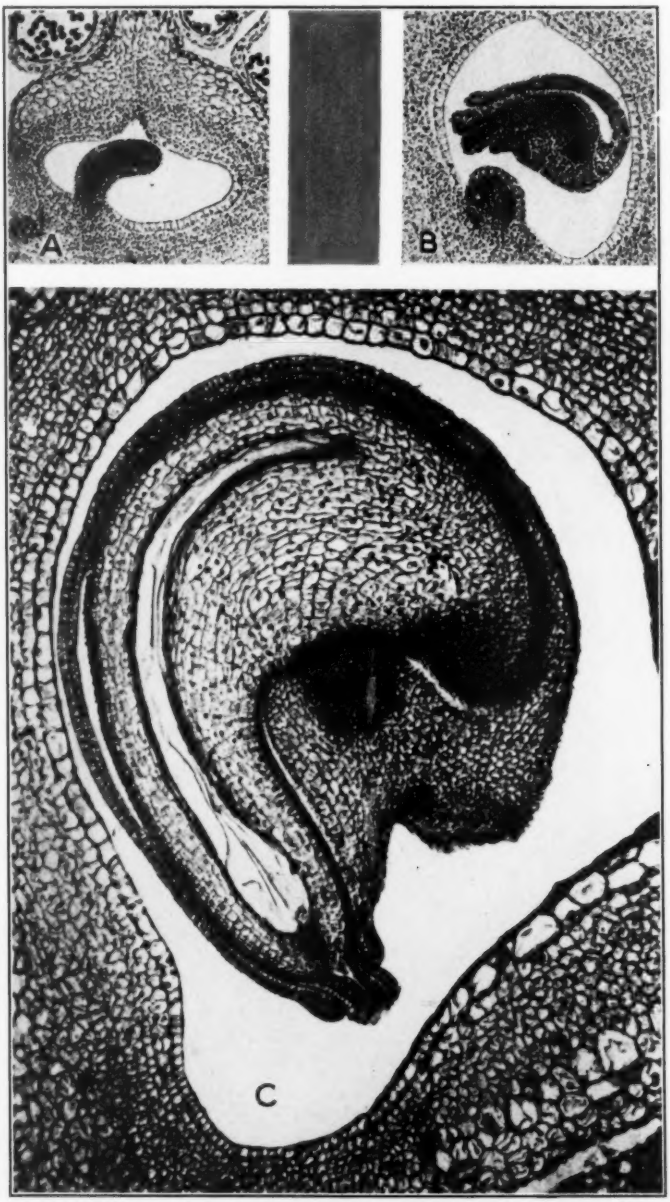


FIG. 9.—A, longitudinal section through ovary with young ovule, × 70; B, cross section through ovary showing the young ovule in longitudinal section, × 94; C, longitudinal section through ovule at an age when the egg is unfertilized, × 60

active pollenizers, a fact that well agrees with the observations of the writer, who invariably found thrips present in flowers examined by him. In countries where thrips do not occur, the wind, as sug-

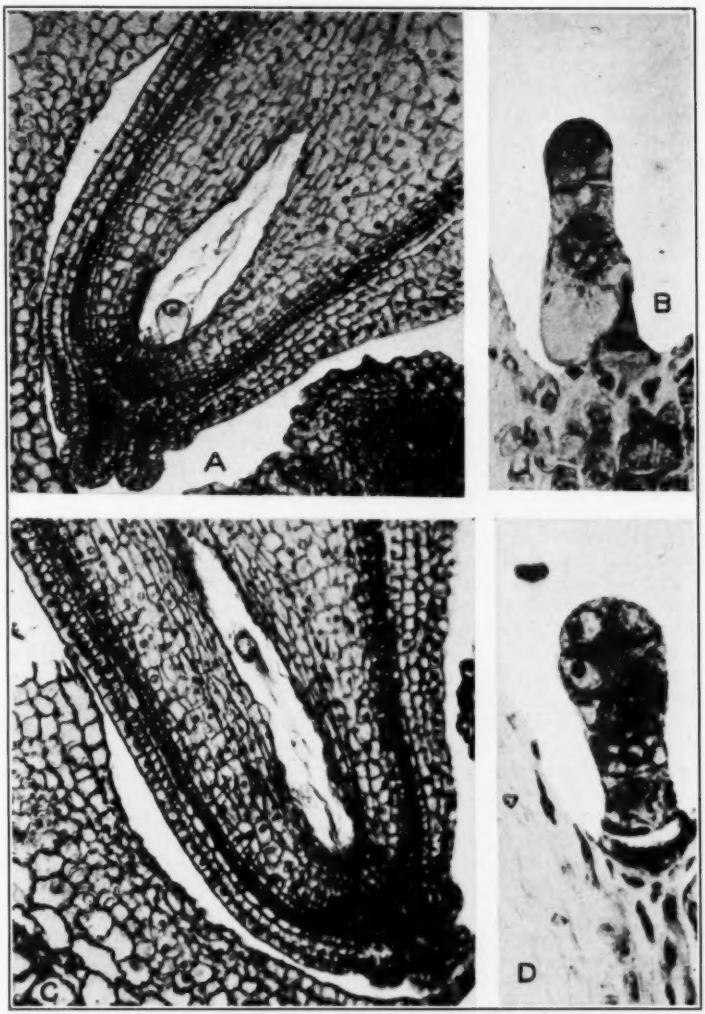


FIG. 10.—A, longitudinal section through micropylar end of ovule, showing the egg nucleus in the embryo sac, × 175; B, 2-celled embryo, × 654; C, different section of the same material as A showing the fused polar nucleus, × 175 D, 6-celled embryo, × 654

gested by Dudok van Heel (*2*), is probably the most effective agent in the dispersal of pollen.

At the time of the opening of the flower the stigmatic lobes are still closed (fig. 11, A). They begin to open very gradually in the after-

noon but may not fully expand until the next day or the following one (fig. 11, B). One frequently observes flowers with anthers already shrivelled but completely closed stigmatic lobes; on the other hand, the shedding of the pollen and the opening of the stigma lobes may occur simultaneously, though such cases are very rare.

The embryo sac at the time of fertilization (fig. 12, A) has only two large nuclei; the egg and the fused polars. The synergids and the antipodals are smaller and at that time are often already in the process of degeneration. When the pollen tube reaches the "egg apparatus" through the micropyle it passes between the synergids and applies itself closely to the egg. The actual discharge of the male nuclei, however, has not been observed. Taking the presence of the pollen tube as evidence that fertilization has occurred, it appears that fertilization takes place at a time when the stigmatic lobes have fully expanded; that is, a day or more after the pollen is shed and that, therefore, normally self-fertilization does not occur. The fertilized egg, immediately or after a brief period of rest, begins to divide to form the embryo. Dudok van Heel (*2*) is of the opinion that the pollen often germinates while the stigmatic lobes are still closed and that fertilization takes place immediately; the fertilized egg, however, rests for several days before it divides again.

The ovule at the time of fertilization is hardly more than 1 millimeter in diameter, but the embryo sac is well developed and digestion of the nucellar tissue proceeds rapidly until the end of the sac arrives in the proximity of the chalaza where it may enlarge somewhat and show an accumulation of nuclei and cytoplasm. After fertilization has occurred, the ovule grows rapidly (fig. 1, B, C, D, E) and attains practically the size of the mature seed before the embryo is fully developed. During this growth of the ovule the sepals of the flower also enlarge and commonly curve back again to a position approximating that of the opening of the flower.

After fertilization the oospore divides horizontally (fig. 12, C) giving rise to two cells (fig. 12, D, fig. 10, B). Soon the lower of the two cells divides again transversely and this is followed by a division of the upper cell. Hereby a tetrad of superimposed cells is formed of which the upper three, to all appearances, give rise to the embryo proper while the fourth develops the suspensor. The latter is filamentous but more than one cell wide.

After the embryo has grown to the 4 or 6 celled stage, the primary endosperm nucleus, formed by the union of the fused polars with the second male nucleus, begins to divide and the nuclei migrate after a manner described by Hegelmaier (*3*) and Dudok van Heel (*2*) toward the periphery, where they become embedded in the cytoplasm and continue to divide (fig. 13, A). At the micropylar end, especially in the vicinity of the lower suspensor cells more divisions occur and the cytoplasm appears more dense. After a period of free nuclear division the endosperm nuclei become invested by walls and gradually the entire cavity of the embryo sac is filled with a delicate tissue (fig. 13, B) which, however, is again dissolved and the content absorbed by the growing embryo with the exception of a single layer of cells surrounding the radicle. This layer becomes distinct from the rest of the endosperm tissue only after the embryo is partly grown (fig. 14). Its cells are small, very regular, and contain a dense granular content.

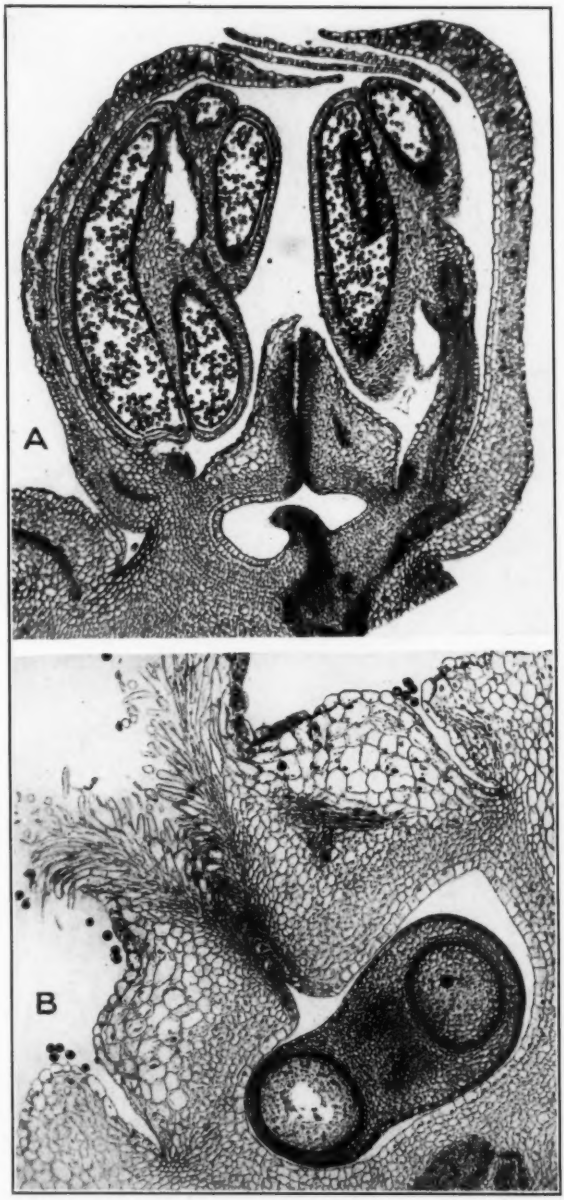


FIG. 11.—A, longitudinal section through a young flower, × 58; B, longitudinal section through an older flower. The ovule is seen in cross section. The stigmatic lobes have spread apart ready to receive the pollen which is seen adhering to the outer surface of the stigma. × 74

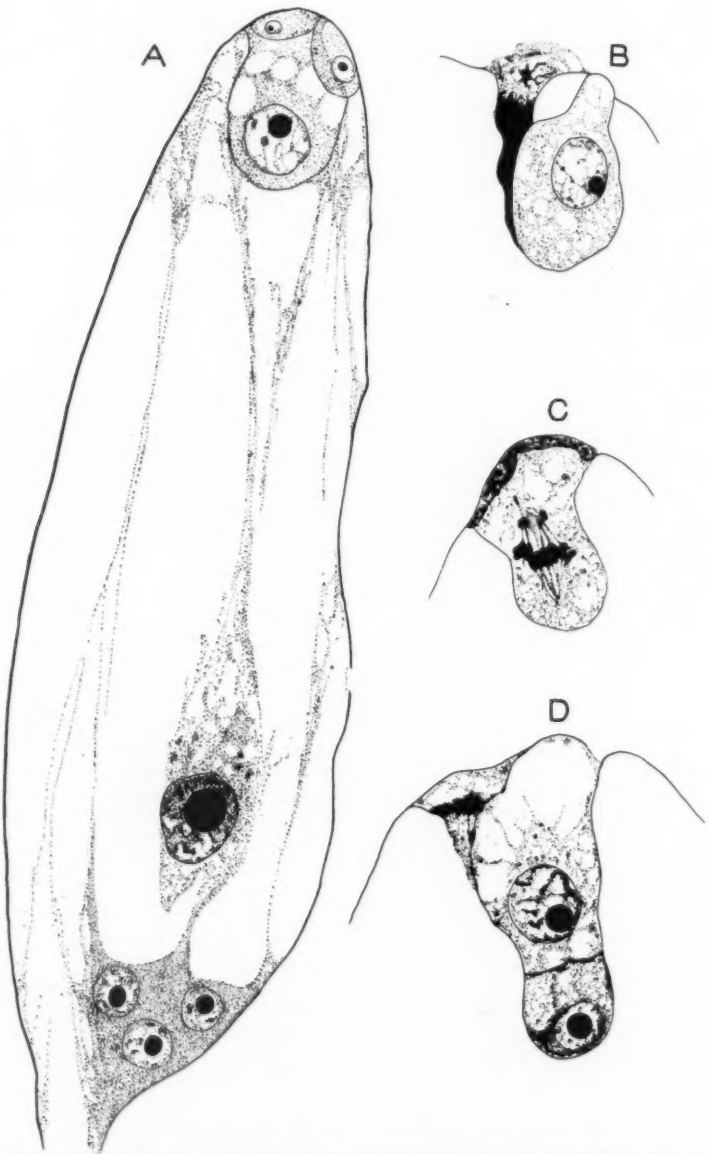


FIG. 12.—A, embryo sac at time of fertilization; B, fertilized egg with pollen tube; C, first division of the fertilized egg; D, 2-celled embryo. All × 923

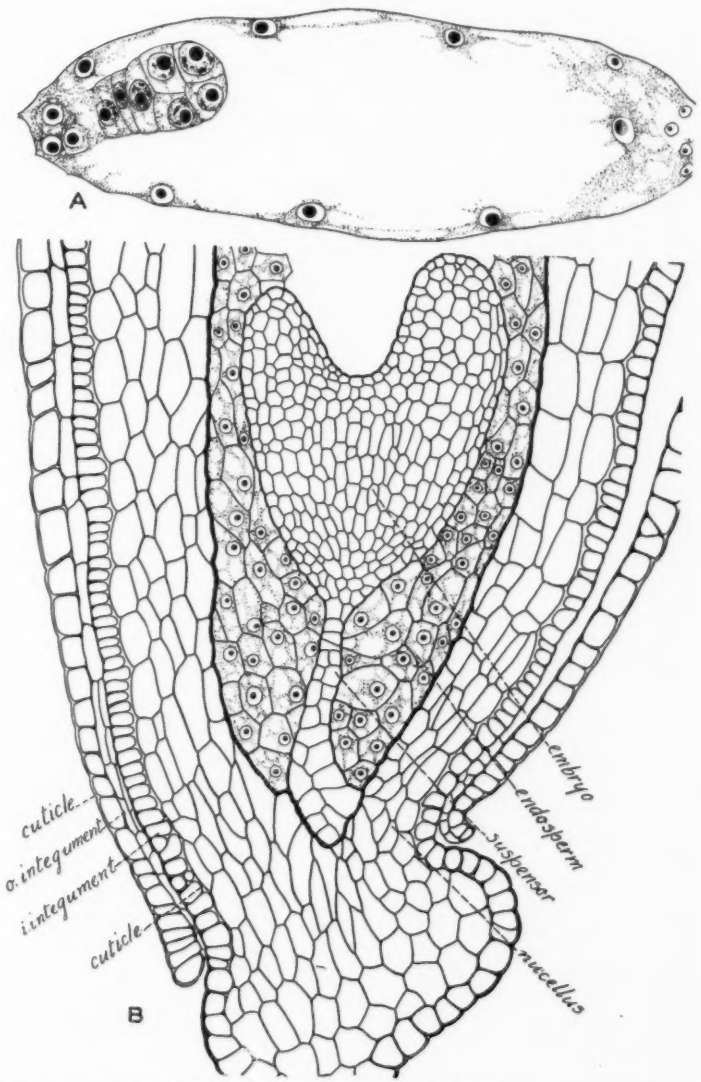


FIG. 13.—A, embryo sac with young embryo and endosperm nuclei, × 470; B, young embryo surrounded by endosperm tissue, × 225

After the embryo sac has reached its final form the peripheral layers of the nucellar tissue are from 4 to 6 cells thick. The embryo and with it the embryo sac enlarges at the expense of the inner peripheral layers which are gradually absorbed. The perisperm at this time contains only a small quantity of starch; soon, however, the cells become packed with large starch grains except in the region of the chalaza, which is always poor in reserve materials.

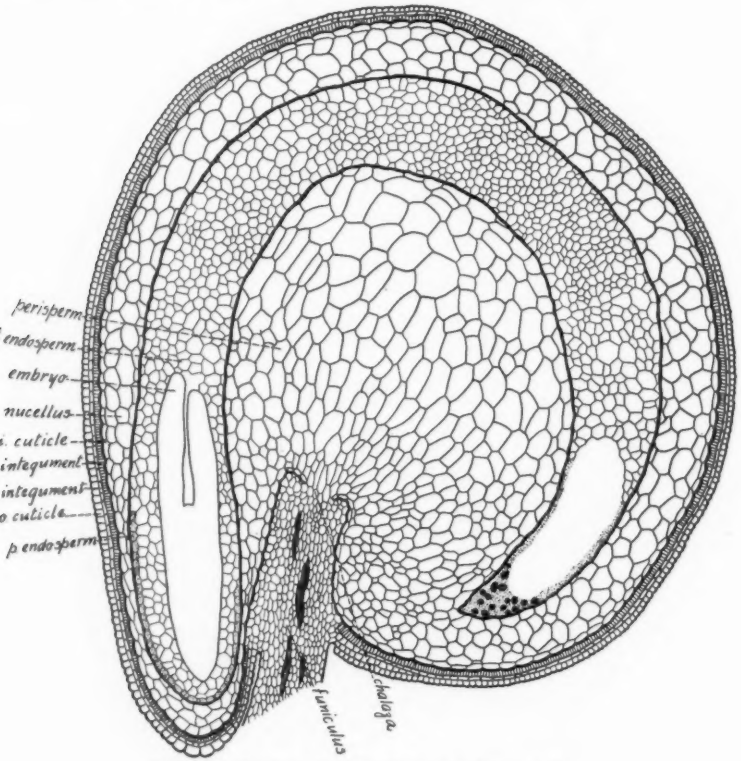


FIG. 14.—Section through ovule with young embryo. × 40

STRUCTURE OF THE SEED AND SEED COAT

The mature seed is a shiny, lentillike structure, about 3 millimeters long and 1.5 millimeters thick (fig. 15, B, and pl. 1, A). The embryo occupies a horizontal position and is curved in such a manner that it completely incloses the perisperm (pl. 1, B). The latter is formed by large isodiametric cells, which in the region of the funiculus appear more or less elongated. The lower part of the radicle is inclosed by a single-layered endosperm.

The mature embryo is protected by a seed coat which has its origin in the integuments of the ovule. The two integuments have two layers each, but the mature organ has only three layers, since the

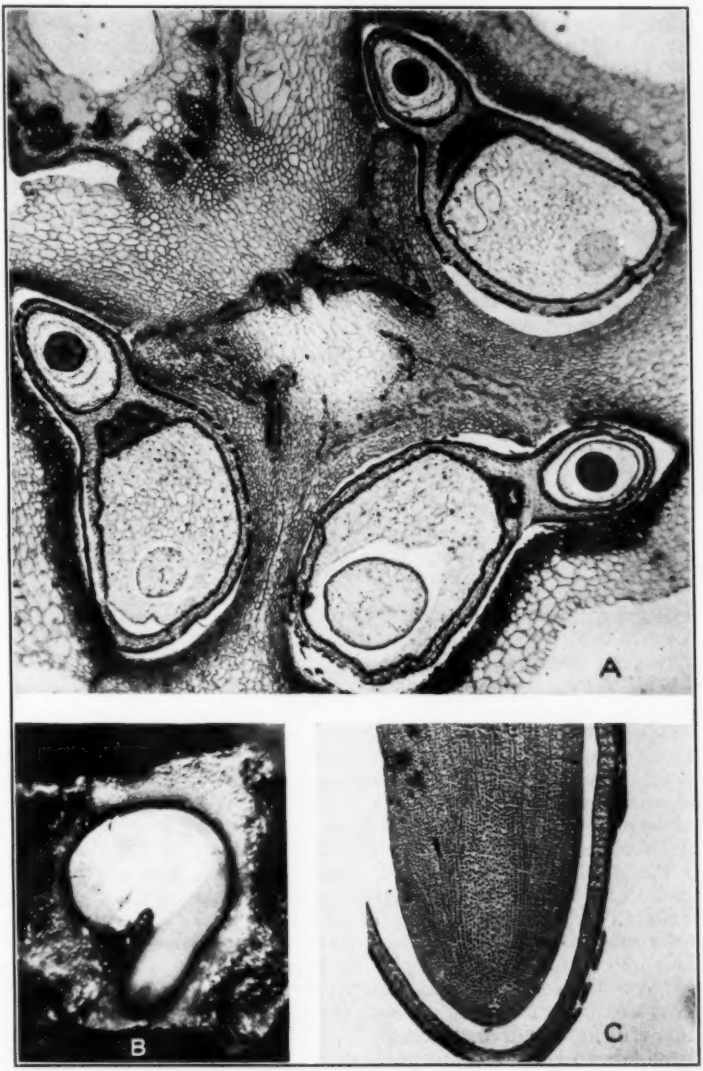


FIG. 15.—A, section through young seed ball showing three ovules with their young embryos in cross section, × 62; B, section through a mature seed, × 13; C, longitudinal section through basal part of embryo. Note the single-layered endosperm surrounding the radicle. × 77

outer layer of the inner integument degenerates during the growth of the seed. The outer integument consists at first of two undifferentiated layers, but the cells of the outer layer early enlarge while their nuclei degenerate. The outer wall begins to thicken and becomes covered by a cuticle which later becomes quite extensive. The second layer consists of small cells with large central nuclei and dense content; the individual cells are separated by small intercellular spaces. The inner integument shows in its meristematic condition nothing that would distinguish it from the outer one, but in its subsequent development it undergoes important changes. The cells of the outer layer remain small and gradually lose their content; the nuclei are obliterated and the layer becomes extinct. The cells of the inner layer enlarge and become delicately sculptured. Between inner integument and nucellus is a thick cuticle which is seen as a wavy line of highly refractive appearance. The cells of the outer integument contain starch and there is an extensive tannin deposit in both integumentary layers (fig. 15, A).

The mature outer seed coat is very brittle and separates easily from the seed. Its reddish brown color is due to the pigmentation of the outer layer. The latter is composed of polygonal, somewhat elongated cells devoid of intercellular spaces. The cells of the inner layer are smaller and when more than one layer thick they appear narrow and elongated. In the mature seed these cells are empty and more or less crushed. The mature inner testa is formed by only a single layer of cells characterized by the above-mentioned striated wall thickenings.

SUMMARY

The microsporangia begin development before the macrosporangia and precede the latter stage for stage. When the pollen is shed the embryo sac is also fully developed, but fertilization does not take place until the stigma lobes have fully expanded, which normally occurs the day after the flowers have opened. For that reason the individual flower of a cluster ordinarily is not selfed.

The nuclear phenomena offer nothing of the unusual compared to that in other plants. The appearance of the chromosomes in diakinesis is regular and there are no unpaired univalents as far as could be determined. The nuclear plate in the heterotypic anaphase contains 9 chromosomes. This number is typical for *Beta maritima* and for most of the sugar-beet material which was available for study. The anaphasic separation is also quite normal, although occasionally there is a lagging of chromosomes. There is a short interkinesis followed by a normal development of the microspores.

The megasporocyte gives rise to four megaspores of which the innermost develops into the embryo sac. The latter possesses the typical number of nuclei. About the time of fertilization the antipodals and synergids degenerate.

The embryo is curved and incloses a perisperm while the radicle is surrounded by a single-layered endosperm, the remains of a tissue which after its formation was absorbed by the developing embryo.

The seed coat consists of an outer and an inner testa derived from the two integuments of the ovule.

The median trace of the floral axis is derived from the left cauline bundle a little distance below the node and it ascends for five internodes before passing out into a leaf. The two lateral traces are also derived from their adjacent cauline bundles; they ascend for two and three internodes, respectively, before entering a leaf. This mode of origin differs from that described by Fron for *Beta cicla*.

LITERATURE CITED

(1) FRON, G.
1899. RECHERCHES ANATOMIQUES SUR LA RACINE ET LA TIGE DES CHÉNOPODIACÉES. Ann. Sci. Nat., Bot. (8) 9: 157–240, illus.

(2) HEEL, J. P. D. VAN
[1925]. ONDERZOEKINGEN OVER DE ONTWIKKELING VAN DE ANTHERE, VAN DEN ZAADKNOP EN VAN HET ZAAD BIJ BETA VULGARIS L. 67 p., illus. Naarden. (Thesis, Delft.)

(3) HEGELMAIER, F.
1885. UNTERSUCHUNGEN ÜBER DIE MORPHOLOGIE DES DIKOTYLEDONEN-ENDOSPERMS. Nova Acta K. Leop. Carol. Akad. Naturf., Bd. 49, no. 1, 104 p., illus.

(4) HÉRAIL, J.
1885. RECHERCHES SUR L'ANATOMIE COMPARÉE DE LA TIGE DES DICOTYLÉDONES. Ann. Sci. Nat., Bot. (7) 2: 203–314.

(5) MATTHYSEN, J. O.
1912. CYTOLOGISCHE UND ANATOMISCHE UNTERSUCHUNGEN AN BETA VULGARIS, NEBST EINIGEN BEMERKUNGEN ÜBER DIE ENZYME DIESER PFLANZE. Ztschr. Ver. Deut. Zuckerindus. (n. F. 49) 62 (2): 137–151, illus.

(6) MEUNIER, A.
1890. LES TÉGUMENTS SÉMINAUX DES CYCLOSPERMÉES. (I PARTIE.) Cellule 6: 299–392, illus.

(7) PAYER, J.–B.
1857. TRAITÉ D'ORGANOGÉNIE COMPARÉE DE LA FLEUR. Text and atlas, 2 v. Paris.

(8) SHARP, L. W.
1926. AN INTRODUCTION TO CYTOLOGY. Ed. 2, 581 p., illus.

(9) SHAW, H. B.
1916. SELF, CLOSE, AND CROSS FERTILIZATION OF BEETS. Mem. N. Y. Bot. Gard. 6: 149–152, illus.

(10) WINGE, Ö.
1917. THE CHROMOSOMES. THEIR NUMBERS AND GENERAL IMPORTANCE. Compt. Rend. Lab. Carlsberg 13: 131–275, illus.

SOME VARIATIONS OF THE HEAT METHOD FOR STERILIZING MILKING MACHINES[1]

By L. H. BURGWALD

Assistant Market Milk Specialist, Bureau of Dairy Industry, United States Department of Agriculture

INTRODUCTION

The method of sterilizing milking machines by the use of heat has been advocated only during the last few years. The chief criticism of this method has been that it is too injurious to the rubber parts of the machines.

REVIEW OF LITERATURE

Bulletin 492 [2] of the New York Agricultural Experiment Station reports experiments carried on by Ruehle in 1917 on the use of hot water for the sterilization of milking machines. In these experiments the teat cups and tubes were washed by drawing cold water, hot alkali water, and clean hot water through them. They were then placed on a stove in a 30-quart covered pail containing water which was heated to 180° to 200° F. The disks and mouthpieces became useless after two days of this treatment. These were replaced, and the temperature was reduced to 160° to 170° F. At this temperature the mouthpieces and disks lasted 17 days, during which time the bacterial counts were very low. At a temperature between 150° and 160° a new set of mouthpieces and disks lasted 25 days, and another set 13 days. Bacterial counts, although still low, were higher than those in the previous tests. Ruehle reached the conclusion that the heat-sterilization method gave very good results bacteriologically but was very destructive to certain rubber parts.

In 1921, Robertson [2] repeated the tests made by Reuhle in 1917, using a temperature of 160° to 170° F. The results were about the same as those obtained by Ruehle. No mention is made of how long the rubber mouthpieces and disks lasted under chemical methods of sterilization. The bulletin mentioned reports that data obtained from control laboratories showed that at least one farm furnishing certified milk in New York State used the heat-sterilization method without undue destruction of the rubber parts.

Reports from laboratory tests conducted in 1918 and 1921 on rubber parts obtained from seven generally used milkers showed that the rubber parts supplied with the majority of milkers were more resistant to heat than was commonly believed. This applied particularly to the best grade of cloth-wrapped, pole-lined, steam-vulcanized tubing, but did not apply so well to the tubing vulcanized in iron molds. It was also noted that in some instances with some metals the rubber apparently melted and stuck to the metal parts.

[1] Received for publication Sept. 2, 1926; issued January, 1927.

[2] ROBERTSON, A. H., FINCH, M. W., and BREED, R. S. MILKING MACHINES: VII. FURTHER STUDIES ON METHODS OF STERILIZATION. N. Y. State Agr. Expt. Sta. Bul. 492, 36 p. 1922.

These tests were made under laboratory conditions only, and the parts were subjected to each of the following conditions daily for 20 days: Under steam pressure in autoclave, in flowing steam, in boiling water, and in hot water at 160° to 170° F. It was not stated whether one method was more or less detrimental than another. The final conclusion was that heat sterilization could be recommended as an effective method for those machines which were, or could be, equipped with rubber parts that would withstand heating twice a day for a reasonable length of time.

In 1920 Hart and Stabler [3] published a report of their experiments in California with the heat-sterilization method. Tests were conducted at two high-class dairies and one ordinary dairy at a temperature of 160° to 190° F. for 15 to 30 minutes. Excellent results were obtained at the two high-class dairies, but the bacterial counts at the ordinary dairy were much higher, running in to the hundreds of thousands. They were lower in number, however, than those obtained before the heat-sterilization method was used. From these tests Hart and Stabler came to the conclusion that "heat sterilization is the only way to sterilize successfully milking machine rubber parts under ordinary ranch conditions." They reported the length of life of the rubber parts at one high-class dairy to be from two to four months, the tubing lasting longer than the inflations.

In 1923, the writer [4] published the results of tests on the heat method of sterilization at temperatures of 160° to 170° F. The parts, after being washed, were submerged in water at these temperatures and allowed to remain undisturbed until the next milking, the water cooling gradually. The results obtained at 20 farms showed the effectiveness of this method of sterilization. The length of life of teat-cup liners ranged from 6 to 17 weeks.

In 1925 the results were published of investigations made by the writer [5] on the comparative value of the heat method, the chlorine method, and the salt and chlorine method of sterilizing milking machines. These tests showed that the heat method, under conditions as nearly identical as possible, gave more uniform and appreciably lower bacterial counts than did the other two methods.

PRESENT INVESTIGATIONS

EXPERIMENTAL METHODS

In order to find a method that would give as good results bacteriologically as the heat method but with an increase in the length of life of the rubber parts, the present investigations were undertaken.

In the heat method referred to, the unit, consisting of teat cups, claw, and tubing, after being washed is placed in hot water at a temperature of 160° to 165° F., and allowed to remain there between milkings, the water cooling gradually.

Three single units were used in these tests. They were washed, sterilized,[6] and handled personally by the writer during the entire

[3] HART, G. H., and STABLER, W. H. EXPERIMENTS WITH AND PRACTICAL APPLICATION OF HEAT STERILIZATION FOR ALL PARTS OF MILKING MACHINES. Jour. Dairy Sci. 3: 33–51. 1920.

[4] BURGWALD, L. H. CLEANING MILKING MACHINES. U. S. Dept. Agr. Farmers' Bul. 1315, 16 p., illus. 1923.

[5] BURGWALD, L. H. CLEANING MILKING MACHINES. Jour. Agr. Research 31: 191–195. 1925.

[6] The term "sterilize" used in this paper indicates a condition of practical rather than of absolute sterilization.

period of each test, each unit receiving exactly the same treatment except for the method of sterilizing or the disposition after sterilization.

Immediately after milking, each unit was rinsed by drawing clean, cold water through by vacuum, washed with a brush in hot water (110° to 120° F.) containing washing powder, and rinsed in clean, hot water. The buckets and heads were rinsed in cold water, washed with a brush in hot water containing washing powder, and rinsed in hot water at a temperature above 160° F. They were again rinsed with hot water (above 160° F.) before using.

The units were taken apart after every sixth to eighth milking, and the rubber parts, consisting of teat cups, claw, and tubing were thoroughly washed. In a few cases they were taken apart only once a week.

EFFECT OF DIFFERENT METHODS OF HANDLING MILKING UNITS ON THE BACTERIAL COUNT OF THE MILK

Throughout one set of tests the units were sterilized as follows:

All the units were placed in a tank of hot water at a temperature ranging from 160° to 167° F., the average temperature being about 163°, and the water was allowed to cool gradually. At the end of 20 to 35 minutes, the average time being 30 minutes, two of the units, Nos. 1 and 2, were removed. No. 1 was placed in a refrigerator, and No. 2 was placed in a weak solution of chlorinated lime. A stock solution was made by dissolving a 12-ounce can of chlorinated lime, containing 24 per cent available chlorine, in 1 gallon of water. This was filtered into a glass bottle, stoppered, and kept in a dark, cool place. The soak solution (about 1:20,000), in which unit No. 2 was kept between milkings, was made by using 1 ounce of the stock solution to 3 gallons of water and was made fresh daily. Unit No. 3 remained in the hot water between milkings.

In winter the temperature of the refrigerator in which unit No. 1 was placed after sterilization ranged from 32° to 48° F., the average being 35°. Temperature readings were taken both when the unit was put in and when it was taken out. The temperature of the weak chlorine solution in which No. 2 was kept between milkings and of the water in which No. 3 was kept ranged from 32° to 62°, the average being 45° at the time the units were removed. The bacterial counts of milk drawn in winter with machines handled as described above are given in Table 1.

TABLE 1.—*Summary of bacterial counts obtained in winter from samples of milk drawn with machines handled in various ways after sterilizing*

Method of handling after sterilizing	Number of samples of milk	Range in bacterial count per cubic centimeter	Average bacterial count per cubic centimeter	Samples having a bacterial count of 10,000 per cubic centimeter or less	
				Number	Per cent
Unit No. 1, placed in refrigerator	188	300 to 10,900	3,130	187	99.5
Unit No. 2, placed in weak chlorine solution	186	200 to 9,300	2,320	186	100.0
Unit No. 3, remaining in hot water	184	300 to 7,400	2,660	184	100.0

In summer the temperature of the refrigerator in which unit No. 1 was placed after sterilization ranged from 34° to 60° F., the average

temperature being 40°. Temperature readings were taken both when the unit was put in and when it was taken out. The majority of readings were 36° to 44°. The temperature of the weak chlorine solution in which No. 2 was kept and of the water in which No. 3 was kept between milkings ranged from 60° to 86°, the average temperature being 73° at the time the units were removed. The bacterial counts of milk drawn in summer with machines handled as here described are given in Table 2.

TABLE 2.—*Summary of bacterial counts obtained in summer from samples of milk drawn with machines handled in various ways after sterilizing*

Method of handling after sterilizing	Number of samples of milk	Range in bacterial count per cubic centimeter	Average bacterial count per cubic centimeter	Samples having a bacterial count of 10,000 per cubic centimeter or less	
				Number	Per cent
Unit No. 1, placed in refrigerator	108	600 to 10,700	3,080	107	99.1
Unit No. 2, placed in weak chlorine solution	106	300 to 5,800	1,990	106	100.0
Unit No. 3, remaining in hot water	106	300 to 5,700	2,410	106	100.0

The results of the winter and summer tests combined are shown in Table 3.

TABLE 3.—*Summary of bacterial counts (Tables 1 and 2 combined)*

Method of handling after sterilizing	Number of samples of milk	Range in bacterial count per cubic centimeter	Average bacterial count per cubic centimeter	Samples having a bacterial count of 10,000 per cubic centimeter or less	
				Number	Per cent
Unit No. 1, placed in refrigerator	296	300 to 10,900	3,110	294	99.3
Unit No. 2, placed in weak chlorine solution	292	200 to 9,300	2,200	292	100.0
Unit No. 3, remaining in hot water	290	300 to 7,400	2,570	290	100.0

There was little difference in the results obtained by the three methods as shown in Tables 1, 2, and 3. All were good. The unit placed in a weak chlorine solution after sterilization gave somewhat lower counts than were secured by the other methods, while the unit placed in the refrigerator gave somewhat higher counts. However, the differences were not significant.

In another set of tests with three units, one unit (No. 4) was sterilized in hot water at a temperature of 145° to 150° F., the average temperature being 148°, and allowed to remain therein between milkings, the water cooling gradually. The other two units (Nos. 3 and 5) were sterilized in hot water at a temperature of 160° to 165°, the average temperature being 162°. At the end of 20 to 45 minutes (average 33 minutes) unit No. 5 was removed, hung in a warm room, and protected from dirt and contamination. Unit No. 3 remained in the hot water between milkings, the water cooling gradually, and was used as a control. The temperature of the room in which unit No. 5 was hung after being removed from the hot water ranged from 70° to 82° F., the average temperature being 75.5° F. The results of these experiments are shown in Tables 4 and 5.

TABLE 4.—*Summary of bacterial counts of samples of milk drawn with units sterilized at different temperatures*

Sterilization temperature	Number of samples of milk	Range in bacterial count per cubic centimeter	Average bacterial count per cubic centimeter	Samples having a bacterial count of 10,000 per cubic centimeter or less	
				Number	Per cent
Unit No. 4, 145° to 150°F	142	3,000 to 51,000	11,930	73	51
Unit No. 3 (control), 160° to 165°F	40	700 to 6,000	2,520	40	100

TABLE 5.—*Summary of bacterial counts of samples of milk drawn with units sterilized at 160° to 165° F., and receiving different subsequent treatment*

Disposition of unit after sterilizing	Number of samples of milk	Range in bacterial count per cubic centimeter	Average bacterial count per cubic centimeter	Samples having a bacterial count of 10,000 per cubic centimeter or less	
				Number	Per cent
Unit No. 5, left in hot water for 20 to 45 minutes, then hung in warm room.	154	1,100 to 24,000	5,540	137	89
Unit No. 3 (control), remaining in hot water	40	700 to 6,000	2,520	40	100

The unit sterilized at 145° to 150° F. gave somewhat higher counts than were obtained by the other methods. The unit heated to 160° to 165° for 20 to 45 minutes and then hung in a warm room gave good results, although somewhat higher counts than those shown in Tables 1, 2, and 3.

As a rule, the units were taken apart and thoroughly washed twice a week, but on a few occasions they were taken apart only once a week. Whether they were taken apart once or twice a week, however, seemed to make little if any difference in the bacterial count of the milk, as is shown in Table 6.

TABLE 6.—*Effect of taking units apart frequently for washing, as shown by bacterial counts of milk drawn with a unit sterilized at 160° to 165° F. and allowed to remain in the water between milkings*

Day No.	Number of milkings since unit was taken apart for washing	Number of samples of milk	Range in bacterial count per cubic centimeter	Average bacterial count per cubic centimeter	Average bacterial count per cubic centimeter for total number of samples on first three and on last three days
1	1	39	400 to 7,400	2,910	
	2	41	700 to 5,400	2,660	
2	3	40	400 to 6,500	2,410	2,580
	4	40	300 to 6,700	2,510	
3	5	36	300 to 6,300	2,410	
	6	36	400 to 6,500	2,570	
4	7	28	700 to 4,700	2,200	
	8	25	1,100 to 5,000	2,540	
5	9	14	900 to 5,900	2,660	2,520
	10	7	1,400 to 5,000	2,590	
6	11	11	1,400 to 5,000	2,860	
	12	12	800 to 6,000	2,740	
	Total	329	300 to 7,400	2,560	

It was noted, however, that the rubber tubing had a tendency to stick to the metal parts at times when the unit was taken apart only once a week.

EFFECT OF DIFFERENT METHODS OF HANDLING MILKING UNITS ON THE LENGTH OF LIFE OF THE RUBBER PARTS

A record was kept of the length of life of the rubber parts when different methods of sterilization were employed. The machine used was of the molded rubber teat-cup liner type. The units were generally used to milk four or five cows each at a milking for two milkings a day. Occasionally, however, they were used for only one milking a day.

The teat-cup liners were the first rubber parts to wear out, the rubber tubing outlasting them considerably. The results of tests to determine the length of life of teat-cup liners under different methods of treatment are given in Table 7.

TABLE 7.—*Length of life of teat-cup liners under various methods of sterilization*

Method of sterilizing	Length of life of teat-cup liners	
	Number of milkings	Number of days allowing two milkings a day
Heat method, 160° to 167° F., remaining in water between milkings	175	87.5
Do	188	94.0
Heat method, 160° to 167° F., 20 to 35 minutes, and placed in refrigerator	315	157.5
Heat method, 145° to 150° F., remaining in water between milkings	218	109.0

As here shown the average life of the teat-cup liners was about three months when sterilized at 160° to 167° F. and allowed to remain in the water between milkings. Removing the unit at the end of 20 to 35 minutes and placing it in a refrigerator increased the length of life of the teat-cup liners to a little over five months. The bacterial counts were almost equally as low as those obtained when the unit remained in hot water between milkings, as shown in Tables 1, 2, and 3.

When a sterilizing temperature of 145° to 150° F. was used and the unit was allowed to remain in the water between milkings, the water cooling gradually, the length of life of the teat-cup liners was increased to 109 days. Bacterial counts of the milk samples drawn with this unit were not so low, however, as those obtained by the other methods. Nearly 50 per cent of the samples had a count of over 10,000 per cubic centimeter, as shown in Table 4.

The short rubber milk tubes on the unit heated to 160° to 167° F. and remaining in the water between milkings, lasted for 332 milkings, or 166 days; while the short air tubes and long milk tube lasted for 432 milkings, or 216 days. The tubing on the machines sterilized by the other methods was still in good condition at the end of 267 days, when the tests were discontinued.

A unit sterilized in a saturated brine and chlorine solution (1:5,000) was also used. The teat-cup liners of this unit were employed for 534 milkings, or 267 days, and were still in excellent condition and probably good for a few more months when the tests were discontinued.

SUMMARY

Heating a unit in water at a temperature of 160° to 167° F. for 20 to 35 minutes and then removing it to a refrigerator and keeping it there between milkings increased the length of life of the rubber parts materially over those left in the hot water between milkings. Bacterial counts on samples of milk drawn with the unit which was placed in the refrigerator were very low, being only slightly higher than those in milk drawn with the unit which was left in the hot water.

Bacterial counts made on samples of milk drawn with a unit sterilized at the same temperature and for the same length of time as the one placed in the refrigerator, but placed in a weak chlorine solution (about 1:20,000) between milkings, were even lower than those obtained with the unit which remained in hot water.

Bacterial counts made on samples of milk drawn with the unit sterilized at a temperature of 160° to 165° F. for 20 to 45 minutes, removed and hung in the warm washroom, and protected from dust and contamination between milkings, were fairly low, although considerably higher than those obtained on milk drawn with the unit which remained in hot water between milkings.

The experiments indicate that sterilizing the units at a temperature of 160° to 167° F. for 20 to 35 minutes and placing them in a weak chlorine solution (about 1:20,000) or hanging them in a refrigerator or cold place (below 50°) protected from dust or contamination, will give excellent bacterial results and that the life of the rubber parts will be materially longer than when the units are allowed to remain in the hot water between milkings. Using the same treatment but hanging the units in a clean, warm place gave low bacterial counts, although not quite so low as those obtained by the other methods.

At a temperature of 145° to 150° F. the life of the rubber parts was somewhat longer than at a temperature of 160° to 165°, but the bacterial counts were not nearly so low as at the higher temperatures.

The rubber tubing always outlasted the teat-cup liners.

The heat method of sterilization or any of its variations shortened the life of the rubber parts more than the salt and chlorine methods.

From a bacteriological standpoint, taking the units apart twice a week seemed to have no advantage over once a week when the heat method was used for sterilizing. The rubber tubing, however, had a tendency occasionally to stick to the metal parts when the units were taken apart only once a week.

19019—27——3

A BIOCHEMICAL STUDY OF THE FALSE-BLOSSOM DISEASE OF THE CRANBERRY[1]

By C. P. SPAETH, *formerly Assistant Chemist*, and H. R. KRAYBILL,[2] *formerly Chemist, Agricultural Experiment Station of the University of New Hampshire*

INTRODUCTION

Although the false-blossom disease of cranberries has been recognized and studied for at least 20 years, its cause remains undetermined. Numerous theories have been advanced to explain its nature, and various control measures have been tried without much success. Lack of drainage, drought, and excessive nitrogen content of the bogs have been blamed for the trouble, but none of these conditions is sufficiently well correlated with the presence of the disease to be regarded as the causal agent. It was thought that a chemical study of diseased and healthy plants might reveal some marked differences in metabolism and perhaps throw more light on whether the disease is due to faulty nutrition. It was hoped further that such a study might lead to a point of attack by which the cause of the disease might be determined and methods for its control or prevention worked out.

DESCRIPTION OF DISEASED PLANT

The disease has been described best by Shear (*10*)[3] in a paper which contains detailed plates and cuts of all stages of the disease. In part, he describes the pathological condition as follows:

"The disease under consideration produces as one of its most conspicuous features a malformation or metamorphy of the floral organs. * * * In the simplest form of the trouble the flower pedicels become more or less erect instead of drooping and the calyx lobes become enlarged, greenish, and somewhat foliaceous. The petals become shortened, broadened, and slightly reddish or greenish in color. * * * The stamens and pistil are more or less aborted and malformed and no fruit is produced.

"All intermediate gradations of phyllody can usually be found among diseased vines, from the simple form, in which there is only a shortening and thickening of the parts of the perianth, to cases in which the entire flower is replaced by a short branch with small leaves.

"Besides the transformation of the floral organs, other abnormalities of growth are usually found. Affected plants have a great tendency to develop lateral branches from the usually latent axillary buds situated on the vine below the fruit bud. * * * The branches are slender and weak and fail to produce normal flowers or fruit. They give the plant a kind of witches'-broom appearance. In some

[1] Received for publication Sept. 1, 1926; issued January, 1927. The material presented forms a part of the dissertation submitted by C. P. Spaeth for the degree of master of science at the University of New Hampshire.

[2] The authors wish to express their appreciation to Neil E. Stevens, Bureau of Plant Industry, United States Department of Agriculture, for suggesting the problem and for much valuable assistance.

[3] Reference is made by number (italic) to "Literature cited," p. 47.

instances the end of the flowering shoot, instead of forming a fruit bud for the next season, as is the case in normal plants, continues to grow and produces a long, slender runner. Cranberry plants in bogs where this malformation occurs generally show an excessive vegetative growth, usually forming a deep, dense mass of vines. In their dormant condition the terminal buds are frequently enlarged and abnormal and die during the winter."

HISTORY AND ECONOMIC IMPORTANCE OF THE DISEASE

The first published records of false-blossom disease appeared in 1908 in a report by C. L. Shear (*9*) read before the Wisconsin Cranberry Growers Association. The disease had been common in Wisconsin for some time previous to that date. It seems to have originated there and to have spread to the other cranberry-growing sections in plantings of Wisconsin nursery stock. In Wisconsin false blossom causes serious loss in production each year. In Massachusetts it is rapidly becoming serious, and in New Jersey also it has gained some headway. An idea of the importance of the disease may be had from a summary of the nursery inspections made in Wisconsin during the years 1915 to 1919. Of 252 acres, the average acreage inspected annually, 54.2 acres, or 21.5 per cent, was found to be infected.

The first report of the disease in the Cape Cod section of Massachusetts was made in 1914, when infected vines were found on five bogs. Although all these plants were destroyed, six new infestations were reported in 1919. In practically all cases where the history of the plantings could be traced it was discovered that the vines originally came from Wisconsin. In 1923, Franklin, of the Massachusetts Cranberry Experiment Station, had a list of 25 infected bogs within a radius of 20 miles of the State bog at Wareham, Mass.

NATURE AND CAUSE OF THE DISEASE

From the very beginning the false-blossom disease was thought to be caused by poor cultural conditions. Shear (*9*) in 1908 pointed out that there appeared to be no fungus connected with the disease, but that "it seems probable that it is due to an unusual disturbance of the physiological functions of the plants, brought about by abnormal conditions of growth and an excessive amount of nitrogenous plant food, derived chiefly from the great quantity of decaying vegetable matter present and associated with an excess of water. These may stimulate a highly abnormal vegetative growth throughout the season and so unbalance the plant that it is unable to produce normal flowers and fruit but instead attempts to change them into vegetative organs." He recommended pruning of the vines, liberal sanding, and better drainage.

Later, in 1914, after making a critical survey of the situation in Wisconsin, Jones and Shear (*5*) came to similar conclusions. At that time they reported that "no evidence exists that the malady is due in any way, directly or indirectly, to the attacks of a parasite, insect, or fungus." They also pointed out that in Wisconsin it does not occur where the best cultural practices are carried out and that the disease disappears when the diseased plants are grown under better cultural conditions. They conclude that apparently the most

important factor in the elimination of false blossom is proper drainage. Shear (*10*) has also observed that where the disease is prevalent there is a deep, coarse peat soil, supplied with excessive water during the greater part of the season. However, Malde (*7*) has noted that the development of the disease is also favored by conditions of extreme drought.

Thus, it appears that the actual cause of the phyllody is still a question to be answered. Certain cultural conditions seem to favor its development, but not consistently enough to be taken as a positive source of the trouble. The transposition of the disease from Wisconsin to other sections of the country leads one to suspect that some organism may be the cause, but thus far no such agent has been found.

As great uncertainty prevails concerning control measures for the disease as concerning the source of the trouble, proper drainage, sanding the bogs, pruning the vines, and, in the most serious cases, scalping and burning over the bogs have been recommended (*10*). Malde (*7*) believes an application of rock phosphate to be beneficial in Wisconsin. However, no one of these remedies, or even all of them, give positive assurance of healthy vines free from false blossom in the future.

In some cases diseased plants that were transplanted and kept under better cultural conditions regained their healthy and fruitful condition. But this recovery was not consistent. It occurred in some cases, while in others the plants remained unfruitful. The disease has been observed on some of the best kept bogs in Massachusetts, in bogs on which the best cultural methods are practiced. It has developed on bogs which have a high moisture content and also on those which have a low moisture content.

MATERIALS AND METHODS OF ANALYSIS

Since the samples were collected in three different years, and since changes and minor improvements were made each year in the methods of sampling, it seems advisable to describe each collection of samples separately.

The samples collected in 1920 were taken by Neil E. Stevens, of the Bureau of Plant Industry, United States Department of Agriculture. Samples of diseased plants were selected from the Vaughn Bog in North Carver, Mass., and those of healthy plants from the Massachusetts State Experiment Station Bog in East Wareham, Mass. All the samples were taken between 10 a. m. and 2 p. m. on clear days. The diseased samples were taken July 14 and 15; the healthy samples July 9 and 11. After collection, the leaves were at once separated from the stems and the weighed samples were placed in hot 95 per cent alcohol containing half a gram of precipitated calcium carbonate to neutralize acidity and were boiled for one hour. The samples were then transported to the New Hampshire Experiment Station by auto and left in the alcohol in quart jars until analyzed.

Samples were again taken in 1922 from the same bogs. That year the samples were all taken the same day, June 28. The diseased plants were sampled between 10 a. m. and noon, and the healthy plants were sampled between 1 and 2 p. m. The samples were brought to the Massachusetts State Cranberry Experiment Station,

where the leaves were at once separated from the stems (the blossoms were included with the leaves), and samples of each were weighed, placed in boiling 95 per cent alcohol containing precipitated calcium carbonate, and boiled for one hour. The samples were taken by auto to the New Hampshire Experiment Station to be analyzed.

In 1923 still more extensive samples were taken. Three bogs infected with the disease were selected, namely, the Cornfield and Thyonnett Bogs, both in East Wareham, Mass., and the Vaughn Bog, in North Carver, Mass. Samples of healthy and diseased plants were taken between 7 and 9 a. m., July 7. The samples were at once brought to the laboratory and the leaves and stems were separated. After the samples had been weighed out they were placed in hot 95 per cent alcohol containing 0.5 gram of precipitated calcium carbonate and boiled for one hour. The stems and leaves were all separated, and by 3 p. m. the samples were preserved in alcohol. The sampling was carried out by members of the Massachusetts Cranberry Experiment Station staff and the New Hampshire Experiment Station staff. The samples were transported by auto to the New Hampshire Experiment Station to be analyzed.

The method of putting up samples described above seems to be the least objectionable for preserving material on which carbohydrate determinations are to be made. In 1920 and 1922 additional samples for tissue analysis were put up. These samples received the same treatment as those already described, except that no calcium carbonate was added.

In determining the carbohydrates, the samples preserved in alcohol containing calcium carbonate were used. These samples, in most cases, consisted of 150 grams of leaves and 75 grams of stems.

The alcohol in which the plant tissue had been preserved was poured off the sample through a filter into a liter volumetric flask. The stems, after being dried in a vacuum oven overnight, were cut with pruning shears into pieces about a centimeter in length. All samples were then removed to 500 c. c. beakers, and just enough 80 per cent alcohol was added to cover them. The beakers were placed on the steam bath, and the liquid was brought to a boil for a few minutes. The beakers were then removed and allowed to come to room temperature, after which the extract was poured through the filter into the liter volumetric flask. This extraction was repeated until the volume of extract was approximately 1,900 c. c. The residue was dried in a vacuum oven at 65° C. and then removed from the beakers for grinding. The empty beakers were cleaned with alcohol, and the filter paper was thoroughly washed into the volumetric flask. The extract was brought to volume at 20° and removed to 2-liter glass-stopped bottles, which were carefully sealed with paraffin.

The residue from the extraction, after being dried in the vacuum oven, was allowed to come to an air-dry condition in the laboratory in dry, weighed beakers. Just before the samples were ground, they were weighed accurately. They were ground very fine in a coffee mill and placed immediately in glass-stoppered bottles. Aliquots of one-fifteenth of the air-dry weights were then weighed into small glass vials for analysis.

In determining the sugars, one-fifteenth aliquots of the residue and extract were taken for analysis. The extract was carefully evap-

orated almost to dryness at a low temperature, to avoid destroying the sugars; it was taken up with a little water; and all the alcohol was driven off. The residue was placed in a beaker, and warm water having a temperature of about 40° C. was added and the dry material thus soaked. This was then placed on a filter and extracted with successive small portions of warm, distilled water, the water extract being added to the beaker containing the evaporated alcohol extract. When the total volume was about 200 c. c. the extract was transferred to a 250 c. c. volumetric flask. The residue on the filter paper was saved for the starch and acid hydrolyzable determinations. The extract was cleared with a small amount of lead acetate, between one-half of 1 c. c. and 1 c. c. being used. After being brought to 20°, the extract was made to volume and the lead precipitate filtered off. The filtrate was then deleaded by the addition of just enough sodium oxalate to precipitate the excess of lead present. This precipitate was filtered off, the solution was brought to 20°, and the reducing sugars were determined.

The reducing power of all of the sugar solutions was determined by a modification of the Bertrand and Munson and Walker methods (*8*). A 25 c. c. aliquot of the cleared solution was used to determine the reducing substances present. A 50 c. c. aliquot of the cleared solution was hydrolyzed by the citric-acid method of Davis and Daish (*2*) for the determination of sucrose. The difference in reduction before and after hydrolysis was calculated and expressed as percentage of dextrose.

The residue left after the water extraction of the samples in the determination of free reducing substances was used for the determination of starch and acid hydrolyzable material. This residue was washed into a 250 c. c. wide-mouthed Erlenmeyer flask with 190 c. c. of distilled water and placed on a boiling water bath for half an hour to gelatinize the starch. The flask was then cooled to 38° C., and 10 c. c. of a 1 per cent solution of takadiastase was added to the solution and mixed thoroughly. A little toluol was then added, and the flask was stoppered and placed in a water bath in which the temperature was accurately controlled at 38°. At the end of 24 hours the flask was removed to a boiling water bath for about 20 minutes to inactivate the enzyme. The solution was then filtered into a 500 c. c. volumetric flask and the residue washed with successive portions of hot distilled water until a volume of 450 c. c. was obtained. The residue was returned to the Erlenmeyer flask and run with new enzyme for another 24 hours to make sure that all the starch had been hydrolyzed. The residue after the second hydrolysis was reserved for the acid-hydrolyzable determination. The filtrate from each hydrolysis was cooled to 20°; a small quantity of neutral lead acetate was added; the filtrate was made up to mark and filtered. To the solution was then added sodium oxalate to precipitate the excess lead, and the lead oxalate was filtered off. Fifty c. c. portions of the filtrate were used in estimating the reducing power due to hydrolyzed starch and the results were calculated and stated as dextrose. A blank experiment for each enzyme was carried through for each set of determinations.

In the determination of acid-hydrolyzable material, the residue from the starch determination was transferred to a 600 c. c. Erlenmeyer flask, to which 142 c. c. of distilled water and 8 c. c. of concentrated hydrochloric acid were added. The flask was then boiled

gently for 2½ hours under a reflux condenser. At the end of that time the solution was filtered into a 500 c. c. volumetric flask and washed with hot water until a volume of 450 c. c. was obtained. This solution was then cooled and neutralized to litmus with sodium hydroxide, and just enough lead acetate was added to clear the solution. The solution was made up to mark at 20° C. It was then filtered, the excess lead was precipitated with sodium oxalate, and the lead oxalate was filtered off. A 25 c. c. aliquot of this solution was used to estimate the reducing power due to acid-hydrolyzable material, and the results were stated as dextrose.

The Kjeldahl method (*1*), modified to include nitrates, was used in determining the total nitrogen.

The phosphorus determinations were made by the Neumann-Pemberton method (*8*).

The method in tissue analysis was based mainly on the procedures employed by Koch (*6*) in his analysis of brain material. The method as outlined is a modification of the original Koch method. It is essentially the separation of the alcohol-preserved material into three subfractions, viz, fraction F_1, or lipoids, fraction F_2, or water soluble, and fraction F_3, or alcohol and water insoluble. Each fraction thus separated was analyzed chemically for the various constituents of interest.

RESULTS

All the analyses presented are the averages of two or three determinations (in most cases made on separate samples) which checked within 5 per cent or less of the total amount of the constituent found.

Table 1 gives the percentage of free reducing substances in the leaves and stems of the diseased and healthy plants. In every case the amount of reducing sugars is higher in the diseased than in the healthy leaves, the difference ranging from 20 to 60 per cent. Similarly, there was a larger amount of reducing sugars in the stems of the diseased plants than in the stems of the healthy plants (except those tested in 1922), the differences ranging from 6 to 37 per cent.

TABLE 1.—*Free reducing substances in tissues of healthy and diseased cranberry plants, expressed as percentages of dextrose*

[Based on fresh weight]

Date of sampling	Source of material	Part of plant	Percentage of free reducing substances			
			In diseased plants	In healthy plants	Excess or deficiency in diseased plants as compared with quantity in healthy plants	Percentage of difference
July, 1920	Vaughn Bog and State bog [a]	Leaves	2.76	2.30	+0.46	+20.00
June, 1922	do.[a]	do.	2.85	2.18	+.67	+30.73
July, 1923	Cornfield Bog	do.	2.85	2.17	+.68	+31.33
Do	Vaughn Bog	do.	3.24	2.39	+.85	+35.56
Do	Thyonnett Bog	do.	3.28	2.04	+1.24	+60.78
July, 1920	Vaughn Bog and State bog [a]	Stems	1.33	1.25	+.08	+6.40
June, 1922	do.[a]	do.	.50	.65	−.15	−23.07
July, 1923	Cornfield Bog	do.	1.07	.83	+.24	+28.91
Do	Vaughn Bog	do.	1.17	.98	+.19	+19.38
Do	Thyonnett Bog	do.	1.14	.83	+.31	+37.34

[a] Healthy plants from the State bog; diseased plants from Vaughn Bog.

The values for sucrose are presented in Table 2. In 1920 there was no difference in the sucrose content of the diseased and healthy leaves, and in 1922 the healthy leaves were higher in sucrose. In 1923, however, on each of the three bogs sampled the diseased leaves were considerably higher in sucrose, the differences ranging from 36 to 113 per cent. In all instances it was found that the diseased stems were higher in sucrose than were the healthy stems, the differences ranging from 28 to 147 per cent.

TABLE 2.—*Sucrose content of tissues of healthy and diseased cranberry plants, expressed as percentage of dextrose*

[Based on fresh weight]

Date of sampling	Source of material	Part of plant	Sucrose content			
			In diseased plants	In healthy plants	Excess or deficiency in diseased plants as compared with quantity in healthy plants	Percentage of difference
July, 1920	Vaughn Bog and State bog [a]	Leaves	0.51	0.51	0.00	0.00
June, 1922	do. [a]	do	1.34	1.74	−.40	−22.98
July, 1923	Cornfield Bog	do	1.83	1.16	+.67	+57.75
Do	Vaughn Bog	do	1.69	.79	+.90	+113.92
Do	Thyonnett Bog	do	1.39	1.02	+.37	+36.27
July, 1920	Vaughn Bog and State bog [a]	Stems	.57	.23	+.34	+147.82
June, 1922	do. [a]	do	.50	.39	+.11	+28.20
July, 1923	Cornfield Bog	do	1.20	.75	+.45	+60.00
Do	Vaughn Bog	do	1.02	.54	+.48	+88.88
Do	Thyonnett Bog	do	1.03	.51	+.52	+101.96

[a] Healthy plants from the State bog; diseased plants from Vaughn Bog.

The results of the starch determinations are given in Table 3. In all the samples except those collected in 1922 the diseased leaves and stems were very much higher in starch than the healthy ones. The starch in the diseased leaves was from 99 to 559 per cent more than the quantity in the healthy leaves.

TABLE 3.—*Starch content of tissues of healthy and diseased cranberry plants, expressed as percentage of dextrose*

[Based on fresh weight]

Date of sampling	Source of material	Part of plant	Starch content			
			In diseased plants	In healthy plants	Excess or deficiency in diseased plants as compared with quantity in healthy plants	Percentage of difference
July, 1920	Vaughn Bog and State bog [a]	Leaves	2.63	1.32	+1.31	+99.24
June, 1922	do. [a]	do	2.45	2.89	−.44	−15.22
July, 1923	Cornfield Bog	do	3.53	.57	+2.96	+519.29
Do	Thyonnett Bog	do	2.90	.44	+2.46	+559.09
Do	Vaughn Bog	do	1.98	.58	+1.40	+241.37
July, 1920	Vaughn Bog and State bog [a]	Stems	.65	.24	+.41	+170.83
June, 1922	do. [a]	do	.10	.80	−.70	−87.50
Do	Cornfield Bog	do	1.82	.90	+.92	+102.22
Do	Vaughn Bog	do	.63	.16	+.47	+293.75
Do	Thyonnett Bog	do	1.16	.38	+.78	+205.26

[a] Healthy plants from the State bog; diseased plants from Vaughn Bog.

Table 4 shows the results of the acid-hydrolyzable determinations. In all cases the diseased leaves and stems are higher in acid-hydrolyzable substances than the healthy ones, the differences ranging from 3 to 77 per cent.

TABLE 4.—*Acid-hydrolyzable substances in tissues of healthy and diseased cranberry plants, expressed as percentage of dextrose*

[Based on fresh weight]

Date of sampling	Source of material	Part of plant	Acid-hydrolyzable content			
			In diseased plants	In healthy plants	Excess or deficiency in diseased plants as compared with quantity in healthy plants	Percentage of difference
July, 1920	State bog and Vaughn Bog [a]	Leaves	4.08	3.42	+0.66	+19.29
June, 1922	do.[a]	do	3.86	3.72	+.14	+3.76
July, 1923	Cornfield Bog	do	5.47	3.09	+2.38	+77.02
Do	Vaughn Bog	do	3.62	2.63	+.99	+37.64
Do	Thyonnett Bog	do	3.08	2.74	+.34	+12.40
July, 1920	Vaughn Bog and State bog [a]	Stems	8.35	7.41	+.94	+12.68
June, 1922	do.[a]	do	5.92	5.48	+.44	+8.02
July, 1923	Cornfield Bog	do	9.84	8.07	+1.77	+21.93
Do	Vaughn Bog	do	8.17	6.38	+1.79	+28.05
Do	Thyonnett Bog	do	9.01	7.01	+2.00	+28.53

[a] Healthy plants from the State bog; diseased plants from Vaughn Bog.

The total carbohydrates are given in Table 5. The figures were obtained by adding the percentages of all of the previously mentioned carbohydrates. In all the samples except those taken in 1922 the diseased leaves and stems contained a larger total of carbohydrates than did the healthy leaves and stems.

TABLE 5.—*Total carbohydrate content of tissues of healthy and diseased cranberry plants, expressed as percentage of dextrose*

[Based on fresh weight]

Date of sampling	Source of material	Part of plant	Carbohydrate content			
			In diseased plants	In healthy plants	Excess or deficiency in diseased plants as compared with quantity in healthy plants	Percentage of difference
July, 1920	Vaughn Bog and State bog [a]	Leaves	9.98	7.55	+2.43	+32.19
June, 1922	do.[a]	do	10.50	10.53	−.03	−.28
July, 1923	Cornfield Bog	do	13.68	6.99	+6.69	+95.71
Do	Vaughn Bog	do	11.45	6.25	+5.20	+83.20
Do	Thyonnett Bog	do	9.73	6.38	+3.35	+52.51
July, 1920	Vaughn Bog and State bog [a]	Stems	10.90	9.13	+1.77	+19.39
June, 1922	do.[a]	do	7.02	7.32	−.30	−4.10
July, 1923	Cornfield Bog	do	13.93	10.55	+3.38	+32.04
Do	Vaughn Bog	do	10.99	8.06	+2.93	+36.35
Do	Thyonnett Bog	do	12.34	8.73	+3.61	+41.35

[a] Healthy plants from the State bog; diseased plants from Vaughn Bog.

The data for the total nitrogen determinations are given in Table 6. In some instances the diseased leaves and stems were higher and in others lower in total nitrogen than the healthy ones. No consistent significant differences in total nitrogen content were found.

TABLE 6.—*Total nitrogen content of tissues of diseased and healthy cranberry plants*

[Based on fresh weight]

Date of sampling	Source of material	Part of plant	Percentage of nitrogen			
			In diseased plant	In healthy plant	Excess or deficiency in diseased plants as compared with quantity in healthy plants	Percentage of difference
July, 1920	Vaughn Bog and State bog [a]	Leaves	0.400	0.484	−0.084	−17.35
June, 1922	do.[a]	do	.404	.383	+.021	+5.48
July, 1923	Cornfield Bog	do	.401	.459	−.058	−12.63
Do	Vaughn Bog	do	.421	.490	−.069	−14.08
Do	Thyonnett Bog	do	.401	.346	+.055	+15.89
July, 1920	Vaughn Bog and State bog [a]	Stems	.310	.284	+.026	+9.15
June, 1922	do.[a]	do	.166	.138	+.028	+20.28
July, 1923	Cornfield Bog	do	.333	.341	−.008	−2.35
Do	Vaughn Bog	do	.334	.311	+.023	+7.40
Do	Thyonnett Bog	do	.293	.212	+.081	+38.21

[a] Healthy plants from the State bog; diseased plants from Vaughn Bog.

The data for the moisture, lipoid substances, water-soluble substances, and insoluble substances are given in Table 7. Moisture is higher in the healthy stems and leaves. The dry matter is lower in the healthy stems and leaves, the differences being distributed among the three fractions of dry matter.

TABLE 7.—*Moisture, lipoid substances, water-soluble substances, and insoluble substances in tissues of diseased and healthy cranberry plants, 1920 samples* [a]

[Based on fresh weight]

Determination	Part of plant	Percentage			
		In diseased plants	In healthy plants	Excess or deficiency in diseased plants as compared with quantity in healthy plants	Percentage of difference
Total moisture	Leaves	56.38	66.36	−9.98	−15.03
Lipoid substances	do	10.73	8.77	+1.96	+22.34
Water-soluble substances	do	8.70	6.99	+1.71	+24.46
Insoluble substances	do	24.18	17.86	+6.32	+35.38
Total moisture	Stems	53.58	55.44	−1.86	−3.35
Lipoid substances	do	5.10	4.85	+.25	+5.15
Water-soluble substances	do	4.07	3.65	+.42	+11.50
Insoluble substances	do	37.22	36.00	+1.22	+3.38

[a] Healthy plants from the State bog; diseased plants from Vaughn Bog.

The amounts of total phosphorus, total ash, water-soluble ash, lipoid ash, and insoluble ash are shown in Table 8. There is no significant difference in the phosphorus content of the healthy and diseased leaves. The healthy stems are slightly higher in phosphorus than the diseased stems. The total ash is highest in the diseased leaves but lower in the diseased stems than in the healthy ones. Water-soluble and insoluble ash were higher in both diseased leaves and stems. The diseased stems and leaves were both lower in lipoid ash.[a]

TABLE 8.—*Total ash, lipoid ash, water-soluble ash, insoluble ash, and total phosphorus content of tissues of diseased and healthy cranberry plants, samples collected in 1920* [a]

[Based on fresh weight]

Determination	Part of plant	Percentage			
		In diseased plants	In healthy plants	Excess or deficiency in diseased plants as compared with healthy plants	Percentage of difference
Total ash	Leaves	1.400	1.300	+0.10	+7.69
Lipoid ash [b] (F_1)	do	.299	.492	−.193	−39.23
Water-soluble ash (F_2)	do	.218	.151	+.067	+44.37
Insoluble ash (F_3)	do	.883	.657	+.126	+19.17
Total phosphorus	do	.045	.044	+.001	+2.27
Total ash	Stems	.903	1.210	−.307	−25.37
Lipoid ash [b] (F_1)	do	.207	.639	−.432	−67.60
Water-soluble ash (F_2)	do	.286	.198	+.088	+44.44
Insoluble ash (F_3)	do	.410	.373	+.037	+9.92
Total phosphorus	do	.047	.056	−.009	−16.07

[a] Healthy plants from the State bog; diseased plants from Vaughn Bog.
[b] By difference, total, $-(F_2+_3)$.

When false blossom of cranberries first became of economic importance in Wisconsin the disease was attributed by Shear (*9, 10*), in part at least, to lack of drainage, whereas Malde (*7*) stated that extreme drought was also responsible in part for the disease. In view of these conflicting opinions it was thought desirable to make a study of the moisture content of some of the bogs on Cape Cod. Samples of soil were collected at various times during the season of 1923 from the State bog, in which there was no false blossom, and from the Vaughn Bog, which was badly infested with the disease. These data are presented in Table 9. Samples of the soil from the State bog were found to be lower in moisture—except those collected on May 26.

Soil samples were also taken from diseased and healthy sections of the same bog at the same time the plants were sampled, in July, 1923. The data are shown in Table 10. This table shows that diseased plants are found on bogs high in moisture and also on those low in moisture content. The soil samples of the diseased section of Vaughn Bog contained 83 per cent moisture, while those from the Thyonnett and Cornfield Bogs contained 36 per cent and 26 per cent, respectively. In two cases out of the three the diseased section of the bog was higher in moisture than the healthy section. These differences, however, are small, varying from 7 to 9 per cent.

Moisture was thought to be an important factor in connection with false blossom because of its supposed effect on the plant's supply of nitrate nitrogen. Therefore nitrate nitrogen was likewise determined on the soil samples collected for the determination of moisture. These data are presented in Tables 9 and 10. Where the moisture is high, as it was in the Vaughn Bog samples, the nitrate nitrogen is in all cases lower. On the other hand, on the Thyonnett Bog, although the moisture is slightly higher in the soil from the diseased section, the nitrate nitrogen is also higher. However, as compared with the quantity of moisture in the Vaughn Bog samples, the moisture of the Thyonnett Bog sample is very low. It is also of interest that no nitrate nitrogen was found in the Cornfield Bog soils.

TABLE 9.—*Moisture and nitrate nitrogen in cranberry-bog soils, 1923*

[The State bog contained healthy plants; Vaughn Bog, diseased plants]

Date of sampling	Percentage of moisture				Milligrams of nitrate nitrogen in 100 grams of soil			
	Vaughn Bog soil	State bog soil	Excess or deficiency in Vaughn Bog soil	Percentage of difference	Vaughn Bog	State bog	Excess or deficiency in Vaughn Bog soil	Percentage of difference
May 26, 1923	59.04	76.54	−17.50	−22.86	0.049	0.189	−0.140	−74.07
June 6, 1923	65.61	52.88	+12.73	+24.07	.189	.483	−.294	−60.87
June 14, 1923	84.35	76.20	+8.15	+10.69	None.	.589	−.589	
June 21, 1923	80.06	76.04	+4.02	+5.28				
July 7, 1923	82.84				.042			
Aug. 15, 1923	81.42	71.36	+10.06	+14.09	.217	.636	−.419	−65.88

TABLE 10.—*Moisture and nitrate nitrogen in soil of healthy and diseased sections of cranberry bogs at time of sampling, July, 1923*

Source of material	Percentage of moisture				Milligrams of nitrate nitrogen in 100 grams of soil			
	Diseased-section soil	Healthy-section soil	Excess or deficiency in diseased-section soil as compared with quantity in healthy-section soil	Percentage of difference	Diseased-section soil	Healthy-section soil	Excess or deficiency in diseased-section soil as compared with quantity in healthy-section soil	Percentage of difference
Vaughn Bog	82.84	73.14	+9.70	+13.26	0.042	0.091	−0.049	−53.84
Thyonnett Bog	36.21	28.58	+7.63	+27.04	.236	.154	+.082	+53.24
Cornfield Bog	25.82	33.02	−7.20	−21.80	None.	None.		

DISCUSSION

It has been suggested (*10*) that the false-blossom disease of the cranberry is due to a disturbance in the physiological function of the plants, due to an excessive amount of nitrogenous plant-food material. The analyses of diseased and healthy plants presented in this paper do not support this explanation. The diseased plants are much

higher in carbohydrates than the healthy plants, and the total nitrogen of the diseased plants is about equal to that of the healthy. If the disease were due to the presence of large amounts of available nitrogen one would expect to find the diseased plants lower in carbohydrates and higher in total nitrogen than the healthy ones.

Improper cultural conditions (*5*, *9*, *7*), leading to poor drainage of the bog, have also been held responsible for the disease. Determinations of the moisture content of the soil of diseased and healthy sections of the bogs show that diseased plants are found in bogs high in moisture and in those low in moisture. While it is probable that cultural conditions may affect the rate of development of the disease and the extent of damage, the bog-moisture data indicate that improper cultural conditions are not the cause of the disease.

From observations (*11*) of the spreading of the disease to Massachusetts and Oregon from Wisconsin through nursery stock, considerable evidence has been presented to show that the disease is infectious. It is probable that the disease may be similar to the virus diseases such as the yellows and the mosaic diseases. It is interesting from this standpoint to compare the composition of healthy and diseased cranberry tissues with those of the healthy and diseased mosaic plants. The very much larger carbohydrate content of the diseased plants is similar to that found in spinach mosaic by True and Hawkins (*12*). No consistent difference was found in the total nitrogen content of the diseased and healthy cranberry plants. Jodidi, Moulton, and Markley (*4*) found the total nitrogen content (oven-dry basis) of the mosaic spinach plant consistently lower than the healthy The healthy cranberry plant differs from the healthy spinach plant, however, in containing a much smaller amount of nitrogen. The spinach plant contains approximately twice as much nitrogen as the cranberry plant. The nonprotein nitrogen of the cranberry plant is exceptionally low.

True, Black, and Kelly (*13*) found the above-ground portions of the healthy spinach plants higher in ash on the dry-weight basis than the diseased. The sum of the ash contents of the cranberry leaves and stems divided by two gives a figure which represents fairly accurately the amount of ash in the entire plant and may be compared with the data on the spinach plant. Recalculating the results obtained upon this basis, it is found that the above-ground portions of the healthy cranberry plants are slightly higher in ash content than the diseased, on the fresh-weight basis, and much higher than the diseased, on the dry-weight basis.

Hartwell (*3*) has pointed out that the lack of sufficient available nitrogen, phosphorus, or available moisture or other conditions which retard growth lead to the accumulation of starch in the above-ground portions of the plant. It has been pointed out, however, that the disease is not due to lack of sufficient available nitrogen or to lack of sufficient moisture. The analyses show no significant difference in phosphorus content of diseased and healthy cranberry tissues. Moreover, numerous fertilizer experiments have shown that the disease can not be prevented by the application of mineral nutrients. In view of these facts it seems very probable that the false-blossom disease of the cranberry may be a virus disease similar to the yellows and mosaic diseases.

SUMMARY

Cranberry plants having the disease false blossom are higher in free reducing sugars, sucrose, starch, acid-hydrolyzable substances, and dry matter and lower in moisture than healthy plants.

No consistent differences were found in the nitrogen content of diseased and healthy plants.

The ash content of the tops of healthy plants is slightly higher than that of the diseased plants.

False blossom of cranberries occurs under widely varying conditions of soil moisture. Improper drainage conditions are not the cause of the disease.

The disease is not caused by excessive quantities of available nitrogen.

It is suggested, as the result of analyses of healthy and diseased plants, that the false-blossom disease of the cranberry may be a virus disease similar to the yellows and mosaic diseases.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1920. official and tentative methods of analysis. as compiled by the committee on revision of methods. revised to nov. 1, 1919. 417 p., illus. Washington, D. C.

(2) Davis, W. A., and Daish, A. J.
1913. a study of the methods of estimation of carbohydrates, especially in plant-extracts. a new method for the estimation of maltose in presence of other sugars. Jour. Agr. Sci. 5: 437–468, illus.

(3) Hartwell, B. L.
1916. starch congestion accompanying certain factors which retard plant growth. R. I. Agr. Expt. Sta. Bul. 165, 23 p.

(4) Joddi, S. L., Moulton, S. C., and Markley, K. S.
1920. the mosaic disease of spinach as characterized by its nitrogen constituents. Jour. Amer. Chem. Soc. 42: 1061–1070.

(5) Jones, L. R., and Shear, C. L.
1914. false blossom of cranberries. Wis. Agr. Expt. Sta. Bul. 240: 55.

(6) Koch, W.
1909. methods for the quantitative chemical analysis of animal tissues. i. general principles. Jour. Amer. Chem. Soc. 31: 1329–1335.

(7) Malde, O. G.
[1910]. report of the cranberry experiment station for the season 1909. Wis. State Cranberry Growers' Assoc. Ann. Rpt. (1909) 22: 12–17.

(8) Mathews, A. P.
1916. physiological chemistry. a text-book and manual for students. Ed. 2, 1040 p. illus. New York.

(9) Shear, C. L.
1908. cranberry diseases in wisconsin. Wis. State Cranberry Growers' Assoc. Ann. Rpt. (1907) 20: 17–21.

(10) ———
1916. false blossom of the cultivated cranberry. U. S. Dept. Agr. Bul. 444, 8 p., illus.

(11) Stevens, N. E.
1925. field observations on false blossom of the cultivated cranberry. Phytopathology 15: 85–91.

(12) True, R. H., and Hawkins, L. A.
1918. carbohydrate production in healthy and in blighted spinach. Jour. Agr. Research 15: 381–384.

(13)——— Black, O. F., and Kelly, J. W.
1918. ash content in normal and in blighted spinach. Jour. Agr. Research 15: 371–375.

THE NITROGEN METABOLISM OF STEERS ON RATIONS CONTAINING ALFALFA AS THE SOLE SOURCE OF THE NITROGEN[1]

By HARRY W. TITUS

Formerly Nutrition Chemist, New Mexico Agricultural Experiment Station

INTRODUCTION

In 1923 the National Research Council's subcommittee on animal nutrition published a list of problems in the field of animal nutrition[2] which they recommended as worthy, in their opinion, of extensive and thoroughgoing study. One of these problems was that of estimating metabolic nitrogen.

The committee proposed the following procedure:

> Choose several proteins which, presumably, are completely digestible. In nitrogen balance experiments add each of these proteins in graduated amounts, in a series of experimental periods, to otherwise nitrogen-free basal rations. That kind of protein which, when thus varied in amount, does not cause the total feces nitrogen to vary we would rate as *completely digestible;* and the total feces nitrogen in these periods would be considered as of metabolic origin.

Shortly before these recommendations were published the writer had prepared plans for a study of the mutual influence of the proportion of the several nutrients in feeds on their digestibility.[3] The procedure outlined was as follows: A 12-pound ration of alfalfa was to be fed to steers for a period of at least two weeks and then two digestion trials, with an interval of at least one week between them, were to be conducted to determine the digestibility of this ration. Then, in place of a part of the original ration, an equal weight of one of the pure nutrients (as nearly pure as it could be obtained) was to be substituted. This modified ration was to be fed for a period of two weeks and then two digestion trials were to be conducted as before. Again, in place of a larger part (twice as large as before) of the ration, an equal weight of the pure nutrient was to be substituted and, after a preliminary two weeks of feeding, two more digestion trials were to be conducted. This modifying of the original ration was to be done four times.

It was planned to study first the effect of crude fiber. As a source of relatively pure crude fiber filter-paper pulp was chosen. Inasmuch as this filter-paper pulp was practically nitrogen free, it will be seen that the writer's procedure was essentially a reversal of the plan recommended by the subcommittee on animal nutrition, in that instead of adding protein to a nitrogen-free ration nitrogen-free material was substituted for an equal weight of a ration relatively rich in protein.

Influenced in part by the recommendations of the subcommittee on animal nutrition, the writer decided to analyze the data obtained

[1] Received for publication Sept. 12, 1926; issued January, 1927.

[2] FORBES, E. B., GRINDLEY, H. S., MORRISON, F. B., ECKLES, C. H., and MOULTON, C. R. PROBLEMS IN THE FIELD OF ANIMAL NUTRITION. Science (n. s.) 57: 567–571. 1923.

[3] GARCIA, F. NUTRITION INVESTIGATIONS. N. Mex. Agr. Expt. Sta. Ann. Rpt. (1922/23) 34: 47. [1923.

to see if they would throw some light on the problem of estimating metabolic nitrogen and on the general problem of nitrogen metabolism. Accordingly, during the course of the investigation and afterwards, the data were so analyzed. The results of this analysis are given in this paper.

EXPERIMENTAL PROCEDURE

The general plan of the investigation has already been given. Five "long yearling" steers [4] were fed for a period of 226 days, and during that time a series of nine digestion trials was conducted with them. Four of the five steers were used in each of the first two digestion trials and three [5] were used in each of the last seven. The digestion trials were 10 days in length and the intervals between them were (*a*) 11 days between digestion trials on the same ration and (*b*) 18 days between digestion trials on different rations. It was during these nine digestion trials that the nitrogen metabolism data herein recorded were secured.

The weight of the feed given daily to each steer was 12 pounds,[5] one-half of this amount at 5 p. m.[6] and the other half at 6.30 a. m. The steers were watered at 3 p. m. and at 10 a. m. Samples of urine and feces were taken each day at 12 m.

Complete details of the investigation from which the data herein given were obtained have been published elsewhere.[7]

COMPOSITION OF THE COMPONENTS OF THE RATIONS, AND OF THE RATIONS

Five different rations were fed; one consisted solely of alfalfa, the other four were definite mixtures of alfalfa and paper pulp. The percentage of each of the two components in each of the five rations was as follows:

	Ration No. 1.	Ration No. 2.	Ration No. 3.	Ration No. 4.	Ration No. 5.
Alfalfa (per cent)	100	85	70	55	40
Paper pulp (per cent)	0	15	30	45	60

The first cutting of alfalfa of very good quality was employed throughout the investigation. The paper pulp used was a high-grade whole-rag commercial product. The composition of the dry matter of the alfalfa and paper pulp is given in Table 1. The composition of the feed used in each of the nine digestion trials is given in Table 2.

[4] Their average initial weight was 586 pounds and their average final weight was 774 pounds.

[5] One of the steers was fed a diminished ration during the last three digestion trials, but the data obtained with this animal during the last three trials were not used in computing the average values herein considered.

[6] The experimental day began at 12 m.

[7] TITUS, H. W. THE MUTUAL INFLUENCE OF THE PROPORTION OF THE SEVERAL NUTRIENTS, IN FEEDS, ON THEIR DIGESTIBILITY. I. CRUDE FIBER—THE DIGESTIBILITY OF RATIONS CONTAINING VARYING AMOUNTS OF ALFALFA AND PAPER PULP. N. Mex. Agr. Expt. Sta. Bul. 153, 52 p., illus. 1926.

TABLE 1.—*Composition of the dry matter of the alfalfa and paper pulp*

ALFALFA

Statistical constants	Ash	Protein	Non-protein	Crude fiber	Ether extract	Nitrogen-free extract	Total nitrogen	Protein nitrogen
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Mean[a]	10.97	14.03	2.12	32.78	1.62	38.48	2.694	2.244
Probable error of mean	±.03	±.06	±.02	±.10	±.01	±.08	±.009	±.009
Standard deviation	.19	.40	.14	.70	.09	.55	.064	.065
Coefficient of variation	1.76	2.88	6.74	2.15	5.49	1.42	2.39	2.88

PAPER PULP

Mean[b]	0.94	0.19	Trace	87.12	0.10	11.64	0.030	0.028
Probable error of mean	±.03	±.02	.....	±.25	±.01	±.25	±.002	±.002
Standard deviation	.20	.09	.....	1.46	.04	1.44	.009	.010
Coefficient of variation	21.08	46.02	.....	1.67	35.87	12.37	29.11	46.02

[a] Computed from the results of 22 separate analyses; the average moisture content of the 22 samples was 8.44 per cent.

[b] Computed from the results of 15 separate analyses; the average moisture content of the 15 samples was 5.67 per cent.

TABLE 2.—*Composition of the feed used in each of the nine digestion trials*[a]

Ration No.	Digestion trial No.	Moisture	Ash	Protein	Non-protein	Crude fiber	Ether extract	Nitrogen-free extract	Total nitrogen	Protein nitrogen
		P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1	1	8.33	10.25	13.64	1.84	29.68	1.59	34.67	2.573	2.182
	2	8.85	9.80	12.71	2.02	29.84	1.54	35.24	2.464	2.034
2	3	8.32	8.64	10.63	1.63	38.06	1.26	31.46	2.047	1.701
	4	9.76	8.17	10.26	1.55	38.52	1.17	30.57	1.971	1.642
3	5	8.32	6.99	8.38	1.55	46.61	.92	27.23	1.672	1.341
	6	7.15	7.05	8.55	1.38	47.07	.96	27.84	1.660	1.368
4	7	6.57	5.90	6.80	1.05	55.50	.67	23.51	1.310	1.088
	8	5.44	6.01	7.21	1.10	55.86	.67	23.71	1.386	1.152
5	9	4.87	4.71	5.06	.79	63.90	.45	20.22	.977	.810

[a] As fed.

THE NITROGEN DATA

Because of the many data involved, only average values of these which pertain to the nitrogen metabolism will be given here. The nitrogen content of the rations and the average nitrogen content of the total excreta, the feces, and the urine are given in Table 3; these same data are presented graphically in Figure 1.

TABLE 3.—*Nitrogen metabolism data (10 days)*

Ration No.	Digestion trial No.	Number of steers averaged	Nitrogen in the feed	Average amount of nitrogen in the total excreta	Average amount of nitrogen in the feces		Average amount of nitrogen in the urine	Average amount of nitrogen retained
					Found	Corrected value[a]		
			Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	1	4	1,400.51	1,379.77	388.00	392.95	991.77	20.74
	2	4	1,331.77	1,331.25	377.60	372.79	953.65	.52
2	3	3	1,114.20	1,085.27	350.01	350.85	735.26	28.93
	4	3	1,072.84	1,049.98	346.99	334.51	702.99	22.86
3	5	3	910.09	833.36	316.72	313.94	516.64	76.73
	6	3	903.56	839.90	332.73	310.87	507.17	63.66
4	7	2	713.05	624.45	340.74	297.01	283.71	88.60
	8	2	754.41	658.56	340.35	303.25	318.21	95.85
5	9	2	508.19	483.96	313.07	256.85	170.89	24.23

[a] Corrected to a uniform water content of 80 per cent by the use of the factor 0.001704 given in equation (1)

METABOLIC NITROGEN

In order to see if the nitrogen content of the feces varied uniformly with the amount of nitrogen ingested, the weight of nitrogen in the feed (for 10 days) was plotted against the average weight of the nitrogen in the feces (for 10 days). (Fig. 1.) All the resulting points did not lie on a straight line; however, deviations from a straight line were concomitant with a high water content of the feces. For this

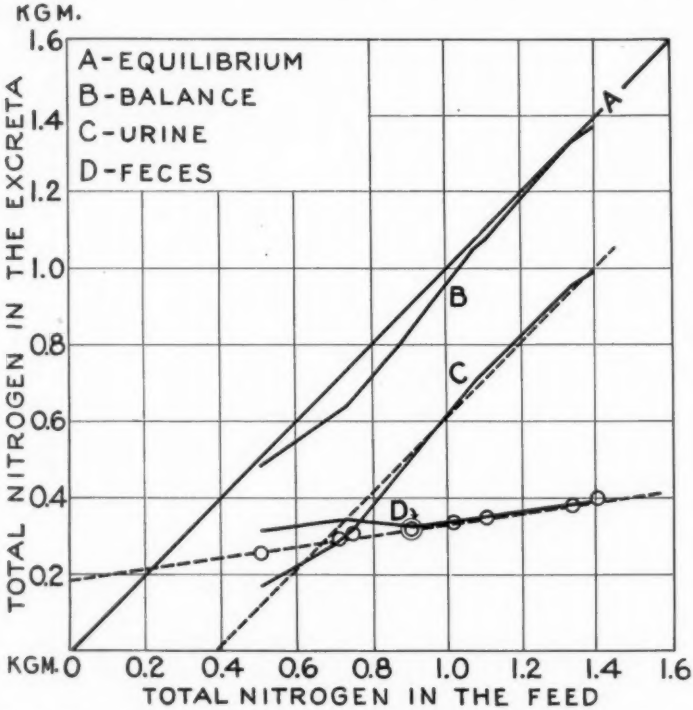


FIG. 1.—Nitrogen metabolism data for 10-day periods. A is the line of nitrogen equilibrium. B is the nitrogen balance curve, that is, the nitrogen in the feed plotted against the nitrogen in the total excreta; the vertical distance between A and B represents the amount of nitrogen retained, if B is below A, and the amount of nitrogen lost, if B is above A. The solid line C results from plotting the nitrogen in the urine against the nitrogen in the feed; the broken line C is a straight line fitted to these plotted points. The solid line D results from plotting the nitrogen in the feces against the nitrogen in the feed; the circles are for the same set of data *after* correcting the nitrogen content of the feces to a uniform water content (80 per cent) of the feces; the broken line D is a straight line fitted to the plotted points indicated by the circles

reason it seemed that the nitrogen content of the feces might be influenced by the water content of the feces. The fact that the above-mentioned points did give a straight line for the first six digestion trials and the additional fact that the slope of this line was much less than would have been observed if the nitrogen in the feed had had the same apparent digestibility throughout, indicated that for each ration there was a more or less fixed amount of nitrogen (metabolic) in the feces which was independent of the true digestibility of the nitrogen of the

feed. Such an amount of nitrogen in the feces would cause the apparent digestibility of the feed nitrogen to become less and less as the amount of ingested feed nitrogen is decreased.

Pfeiffer [8] corrected for the nitrogenous excretory products by subtracting 0.4 gm. of nitrogen per 100 gm. of digested dry matter. Assuming that the quantity of metabolic nitrogen in the feces is dependent upon the amount of digested dry matter and upon the amount of water in the feces, an attempt was made to determine the empirical relationship. As a result of this attempt, the following equation for determining the amount of metabolic nitrogen in the feces was obtained:

$$m = 0.002813a + 0.001704b \quad (1)$$

in which

m = weight of metabolic nitrogen in the feces,
a = weight of dry matter digested, and
b = weight of water in the feces.[9]

If it is assumed that the metabolic nitrogen in the feces is dependent upon the dry matter consumed and the water content of the feces, rather than upon the dry matter digested and the water content of the feces, the following relationship is obtained:

$$m = 0.002428a_1 + 0.001562b \quad (2)$$

in which

m = weight of metabolic nitrogen in the feces,
a_1 = weight of dry matter consumed, and
b = weight of water in the feces.

When the average digestibility of the nitrogen in the alfalfa is computed, after correcting for metabolic nitrogen, it is found to be 88.16 per cent if correction is made according to equation (1), and 90.13 per cent if correction is made according to equation (2).

It can not be said, as yet, which equation will give more nearly the correct amount of metabolic nitrogen in the feces. Application of the two equations and further investigation should give some indication as to which set of assumptions is more nearly correct and to what extent the equation dependent upon that set of assumptions may be relied upon. The writer gives preference to equation (1).

As a check on the previously mentioned indication that the water content of the feces influences its nitrogen content, the nitrogen content of the feces was corrected [10] to a uniform water content of 80 per cent. The resulting values were plotted against the nitrogen

[8] According to Armsby. ARMSBY, H. P. THE NUTRITION OF FARM ANIMALS. p. 120. New York. 1917.

[9] In determining the constants in equation (1), an equation similar to the following was set up:

$$ax + by - nz + d = 0$$

in which

a = weight of the dry matter digested from the feed,
x = weight of metabolic nitrogen dependent upon unit weight of digested dry matter,
b = weight of water in the feces,
y = weight of metabolic nitrogen dependent upon unit weight of water in the feces,
n = weight of total nitrogen in the feed eaten,
z = the "true" digestibility of the feed nitrogen, and
d = the difference between the weight of feed nitrogen and the weight of fecal nitrogen.

This equation, then, expresses the following relationship: The nitrogen not excreted in the feces (d) is equal to the total amount of nitrogen digested (nz) minus the metabolic nitrogen ($ax + by$).

The proper values of a, b, n, and d (for each digestion trial) were then substituted in this equation; thus, nine equations in the three unknowns were obtained. The values of x, y, and z were then determined by the method of least squares. They were found to be 0.002813, 0.001704, and 0.8816, respectively.

The values of the constants in equation (2) were obtained in a similar manner.

[10] By the use of the factor 0.001704, given in equation (1).

content of the feed and all the points (indicated by circles in fig. 1) were found to lie very nearly along a straight line. This would indicate that the water content of the feces does influence its nitrogen content.

The equation of this straight line, determined by the method of least squares,[11] was found to be:

$$y = 0.1447x + 184.95 \quad (3)$$

in which

y = weight (gms.) of nitrogen in the feces (10 days) after being corrected to an 80 per cent water content, and
x = weight (gms.) of nitrogen in the feed (10 days).

This line intersects the y-axis at 184.95 and hence this value is the weight of metabolic nitrogen which the feces (for 10 days) would contain if the ration (12 pounds) contained only paper pulp, i. e., if the ration were nitrogen-free. This is appreciably less than the amount of metabolic nitrogen (219.14 gm.—average value) estimated as resulting from the ingestion of a 12-pound ration of alfalfa. The conclusion follows that the amount of nitrogen in the feces of a steer consuming a nitrogen-free ration may not safely be taken as a measure of the amount of metabolic nitrogen resulting from the ingestion of an equal weight of alfalfa, or other feeding stuff.

THE "TRUE" DIGESTIBILITY OF THE NITROGEN IN ALFALFA

If we correct the nitrogen content of the feces, by subtracting the amounts of metabolic nitrogen estimated by the use of equation (1), and then compute the average "true" digestibility, we find, as stated before, that it is 88.16 per cent. The average "true" digestibility of nitrogen, for each digestion trial, is as follows:

Trial No. 1	Trial No. 2	Trial No. 3	Trial No. 4	Trial No. 5	Trial No. 6	Trial No. 7	Trial No. 8	Trial No. 9
87.62	88.45	87.99	88.65	88.43	88.88	87.65	87.73	88.03

The mean value is 88.16; the probable error of the mean is ± 0.10; the standard deviation is ± 0.43; and the coefficient of variation is 0.49 per cent.

Realizing that the above method of calculating the extent of the variation (i. e., by using average values) has a tendency to decrease the probable error of the mean, the standard deviation, and the coefficient of variation, the "true" digestibility of the nitrogen in the ration of each steer during each digestion trial was computed and, from these 29 values, the above mentioned statistical constants were recalculated. The following values were thus obtained: Probable error of the mean, ± 0.39; standard deviation, ± 2.03; and coefficient of variation, 2.31 per cent. The greatest variations were observed during the last three digestion trials; for the first six trials the mean (of 20 values) was 88.31, the probable error of the mean was ± 0.16, the standard deviation was ± 1.08, and the coefficient of variation was 1.22 per cent.

Thus it is seen that rather concordant values of the "true" digestibility of the nitrogen in alfalfa were obtained when correction was made by the use of equation (1).

[11] The average values were weighted according to the number of steers used in securing them.

RELATION BETWEEN NITROGEN CONTENT OF THE URINE AND NITROGEN CONTENT OF THE TOTAL EXCRETA

When the nitrogen content of the urine was plotted against the nitrogen content of the total excreta, all the resulting points lay very nearly upon a straight line. The equation of this straight line was determined by the method of least squares [11] and found to be:

$$y = 0.9171x - 266.56 \quad (4)$$

in which:

y = weight (grams) of nitrogen in the urine (10 days), and
x = weight (grams) of nitrogen in the total excreta (10 days).

It should be noted that equation (4) holds only when the weight of the ration is 12 pounds. When the weight is not 12 pounds, the constant 266.56 will have a different value. For other weights the following equation is tentatively suggested:

$$y = 0.9171x - 22.21R \quad (4a)$$

in which R is the weight of the ration in pounds.

As a check on the constancy of this relationship the nitrogen content of the urine was calculated, by means of equation (4), from the corresponding nitrogen content of the total excreta. These calculated values, together with the average values found and the difference between the found and calculated values, are given in Table 4. The found and calculated nitrogen content of the urine of the individual steers when consuming a 12-pound ration of alfalfa is given in Table 5. The indications are that this relationship between the nitrogen content of the urine and the nitrogen content of the total excreta is very constant when alfalfa is the source of the nitrogen.

TABLE 4.—*The found and calculated nitrogen content of the urine (10 days), by digestion trials*

Ration No.	Digestion trial No.	Number of steers averaged	Average weight of the nitrogen in the urine			
			Found	Calculated	Absolute difference	Proportional difference
			Gm.	*Gm.*	*Gm.*	*Per cent*
1	1	4	991.77	998.83	−7.06	0.71
	2	4	953.85	954.51	−.66	.07
2	3	3	735.26	728.74	+6.52	.89
	4	3	702.99	696.38	+6.61	.95
3	5	3	516.64	497.71	+18.93	3.80
	6	3	507.17	503.71	+3.46	.69
4	7	2	283.71	306.12	−22.41	7.32
	8	2	318.21	337.41	−19.20	5.69
5	9	2	170.89	177.08	−6.19	3.50

[11] The average values were weighted according to the number of steers used in securing them.

TABLE 5.—*The found and calculated nitrogen content of the urine (10 days) of the individual steers when consuming a 12-pound ration of alfalfa*

Digestion trial No.	Steer No	Weight of the nitrogen in the urine			
		Found	Calculated	Absolute difference	Proportional difference
		Gm.	*Gm.*	*Gm.*	*Per cent*
1	42	961.31	985.66	−24.35	2.47
	43	966.96	976.18	−9.22	.95
	44	1,023.27	1,022.72	+.55	.05
	45	1,015.53	1,010.75	+4.78	.47
2	42	924.86	950.40	−25.54	2.69
	43	960.91	966.29	−5.38	.56
	44	957.89	948.70	+9.19	.97
	45	970.96	951.92	+19.04	2.00
Average					1.27

URINARY NITROGEN AND THE NITROGEN REQUIREMENT FOR MAINTENANCE

When the average nitrogen content of the urine (for 10 days) was plotted against the nitrogen content of the feed, the resulting points (fig. 1) indicated a more or less linear relationship. The equation of the straight line fitted to these points was found to be:

$$y = 0.9937x - 383.69 \qquad (5)$$

in which

y = weight (gms.) of nitrogen in the urine (10 days), and
x = weight (gms.) of nitrogen in the feed (10 days).

This equation represents, at least approximately, the manner in which the urinary nitrogen decreased as the feed nitrogen was decreased. The x-intercept [12] of the line drawn according to the above equation is 386.14. However, a steer consuming 386.14 gm. of nitrogen in its feed, per 10 days, would excrete an appreciable amount of nitrogen in its urine, and so it is clear that equation (5) can not represent conditions when the nitrogen intake approaches and becomes less than that required for maintenance.

Had this investigation been conducted with mature steers, it seems possible that the x-intercept of this line [13] might represent the animals nitrogen requirement for maintenance. If, in this case, the x-intercept be taken as the average amount of nitrogen required for the maintenance of each steer during the last three digestion trials, we find that 386.14 gm. of nitrogen was required to maintain a 780-pound steer (average weight) for 10 days; this is equivalent to 0.493 pound of digestible[14] crude protein, per day, per 1,000 pounds average live weight. This value is distinctly lower than the value given by Armsby,[15] but within the range of variation he suggested for his value.

[12] The point on the line corresponding to a urinary nitrogen excretion of zero.
[13] The points fixing this line would have to be determined by nitrogen intakes well above the maintenance requirements.
[14] *Apparent* digestibility, 71.97 per cent.
[15] ARMSBY, H. P. THE NUTRITION OF FARM ANIMALS. p. 327. New York. 1917.

THE NITROGEN BALANCES

In Figure 1 the nitrogen in the feed was plotted against the nitrogen in the total excreta and the line connecting the resulting points was called a "balance curve." The amount of nitrogen retained is represented by the vertical distance between the balance curve and the equilibrium line.

One point worthy of observation is that the nitrogen balances became larger and larger as the amount of paper pulp in the ration increased [16] until it amounted to 45 per cent; after that the nitrogen balances decreased. The only explanation offered (and it does not seem wholly satisfactory) is that the available energy of the ration was the limiting factor during the first part of the investigation and that during the latter part (when the ration contained only 40 per cent alfalfa) the nitrogen of the ration was the limiting factor. This explanation involves the assumption that, weight for weight, the paper pulp supplied an appreciably greater amount of available energy than did the alfalfa. In view of the high apparent digestibility (77.56 per cent [17]) of the paper pulp, the above assumption could easily be true.

CONCLUSIONS

The results of the analysis of the data given in this paper seem to warrant the following conclusions:

(1) The amount of metabolic nitrogen in the feces of a steer is influenced, among other things, by the water content of the feces.

(2) A linear relationship exists between the nitrogen content of the feces of a steer (when corrected to a uniform water content) and the nitrogen content of its feed, when the latter is decreased by substituting paper pulp (probably any digestible carbohydrate material) for an equal weight of a given ration of alfalfa.

(3) The fecal nitrogen excretion of a steer consuming a nitrogen-free ration can not safely be taken as a measure of the metabolic nitrogen in the feces of the animal when consuming an equal weight of a given feeding stuff.

(4) The "true" digestibility of the nitrogen of alfalfa is approximately 88 per cent.

(5) A linear relationship exists between the nitrogen content of the urine and the nitrogen content of the total excreta of a steer, if the source of the nitrogen is alfalfa, when the nitrogen content of the feed is varied as it was in this investigation.

(6) When the nitrogen in the feed is varied, as it was in this investigation, an approximately linear relationship exists between the nitrogen content of the urine and the nitrogen content of the feed, if the animal, at all times, is receiving appreciably more nitrogen than it requires for maintenance. With a mature steer (possibly with a

[16] This is similar to the experience of Wicke and Weiske, who added 174 gm. of starch to a basal ration containing 800 gm. of hay and 200 gm. of flaxseed and observed an increase of the nitrogen balance (with sheep) from 0.96 gm. to 1.64 gm. per day. WICKE, A., and WEISKE, H. ÜBER DEN EINFLUSS EINER FETT- RESP. STÄRKEBEIGABE AUF DIE AUSNÜTZUNG DER NÄHRSTOFFE IM FUTTER UND AUF DEN N-UMSATZ UND ANSATZ IM THIERKÖRPER. (2. VERSUCHSREIHE.) Hoppe-Seyler's Ztschr. Physiol. Chem. 22: 137–152. 1896.

[17] TITUS, H. W. THE MUTUAL INFLUENCE OF THE PROPORTION OF THE SEVERAL NUTRIENTS, IN FEEDS, ON THEIR DIGESTIBILITY. I. CRUDE FIBER—THE DIGESTIBILITY OF RATIONS CONTAINING VARYING AMOUNTS OF ALFALFA AND PAPER PULP. N. Mex. Agr. Expt. Sta. Bul. 153, 52 p., illus. 1926.

growing steer) this relationship may be of value in estimating the amount of nitrogen the steer requires for maintenance.

(7) The substitution of paper pulp (within certain limits) for an equal weight of alfalfa, in a ration consisting solely of alfalfa, causes an increased retention of nitrogen. It is highly probable that this effect of the paper pulp is dependent upon the size of the original alfalfa ration.

INJURY TO GROWING CROPS CAUSED BY THE APPLICATION OF ARSENICAL COMPOUNDS TO THE SOIL[1]

By H. E. MORRIS, *Associate Botanist and Plant Pathologist*, and D. B. SWINGLE, *Botanist and Bacteriologist, Montana Agricultural Experiment Station*

INTRODUCTION

In a preliminary paper (*14*) [2] brief mention was made of the report of Headden (*8*) on the injury to crowns of fruit trees in Colorado caused by the use of arsenicals, and a detailed outline was given of a series of experiments made by the writers for the purpose of determining whether the arsenical compounds commonly used as insecticides are injurious to the bark of fruit trees, the nature and extent of injury, the relative toxicity of different arsenicals, and the conditions most favorable to injurious action.

The work here recorded, which was done between 1911 and 1922, at Bozeman, Mont., and at Madison, Wis., presents another phase of the general problem of the effect of arsenical compounds on plant life, but deals primarily with the results obtained by adding the chemical to the soil.

HISTORICAL REVIEW

A review of early literature reveals the fact that arsenical compounds, especially arsenic trioxide, were known in ancient times. Arsenic was early recognized as a typical poison for plants as well as animals, and its effects upon plant life have been the subject of investigation for many years.

De Candolle (*3, p. 1328–1332*) in 1832 refers to the work of Jaeger, Marcet, and Macaire and reviews in considerable detail the work of Leuchs, who found a decrease in dry weight of vetch plants when grown in the presence of arsenic trioxide.

Chatin (*4*) discovered that arsenic absorbed by the roots was distributed to various tissues of plants.

Davy (*1*) concluded from his work on the effect of arsenic on growing plants: (1) That plants, at least peas and turnips, are not injured by arsenic in the soil; (2) that they absorb this dangerous element; and (3) that they retain it in their tissues.

Daubeny (*5*) watered barley plants five times in succession with a solution of arsenious acid, 1 ounce in 10 gallons of water, and found that the crop matured a fortnight earlier, but that the amount of grain harvested was less than the normal yield. Four waterings of turnips with the same solution did not hasten maturity, but slightly decreased the yields. Analysis did not indicate any arsenic in the tissues.

Gorup (*7*) grew to maturity plants of *Polygonum fagopyrum*, *Pisum sativum*, and *Secale sativum* in soil containing arsenious acid, 30 gm. of the acid in 30.7 cu. decim. of soil, each unit growing two plants which matured normally. Analysis by Marsh's test showed

[1] Received for publication June 1, 1926; issued January, 1927.
[2] Reference is made by number (italic) to "Literature cited," p. 77.

no trace of arsenic in 20 gm. of dry matter of *Secale cereale*, but in 148 gm. of *Polygonum fagopyrum* a weak mirror was formed. He concluded that these plants, especially *Pisum sativum*, are indifferent to relatively large quantities of arsenious acid in the soil.

Freytag (*6*) found that one-eightieth per cent of arsenious acid in water culture solution was fatal to beans, peas, and other plants, and he concluded that plants have not a selective power since they absorb poisonous as well as nutritive substances.

McMurtie (*10*) concluded from his work that plants have not the power to absorb and assimilate compounds of arsenic from the soil but that such compounds may exert an injurious influence upon vegetation, although not until the quantity present reaches in the case of Paris green about 900 pounds per acre, in the case of arsenite of potash about 400 pounds per acre, and in the case of arsenate of potash about 150 pounds per acre.

Phillips (*12*), experimenting with greenhouse plants, found that calcium arsenate when present in the soil in toxic amounts checked the formation of roots to such an extent as to interfere with nutrition and growth, or else it killed the plant outright. Analyses showed no traces of arsenic in the poisoned plants.

Nobbe, Baessler, and Will (*11*) found that one part of arsenic in one million parts of water had an injurious effect on buckwheat, oats, maize, and peas when grown in water culture.

Blyth (*2*) stated that if plants are poisoned with arsenic, the toxic action may be traced from below upwards, and analyses will detect minute quantities of arsenic in all parts of the plant.

Lyttkens (*9*) found that the addition of 0.005 to 0.01 per cent of arsenious acid (as the potassium salt) to garden soil in which barley was growing caused a feeble growth and a blue-green color.

In regard to the occurrence of arsenic in plant tissues, a great deal of work has been done, and the general conclusion has been reached that arsenic is quite generally a constituent of plant tissue when plants are grown in its presence.

Wanklyn (*16*), discussing arsenic, says that "minute traces of arsenic are all-pervading, and, as necessary consequence, the mere detection of arsenic is devoid of meaning unless it is, to some extent, a quantitative operation."

DESCRIPTION AND RESULTS OF EXPERIMENTS

TRANSPIRATION STUDIES WITH OAT PLANTS IN WATER CULTURES

A group of preliminary experiments was made with oat plants in water cultures.

The containers used were ordinary wide-mouthed flint glass bottles of approximately 530 c. c. capacity, covered with a uniform coat of varnish. They were cleaned with a sulphuric-potassium-chromate cleaning fluid and thoroughly rinsed with distilled water. The water was obtained by double distillation; its conductivity, tested at different times, varied from 4 to 6 times 10^{-5} ohms. The chemicals of a high degree of purity, were as follows: Arsenic trioxide, by analysis 73.1 per cent arsenic; calcium nitrate; ferric chloride; magnesium sulphate; potassium phosphate (monobasic); Shive's (*13*) 3-salt nutrient solution No. R5C2, which contained potassium phosphate, 0.018 m., calcium nitrate, 0.0052 m., magnesium sulphate,

0.0150 m; and ferric chloride 0.068 per cent solution, 5 c. c. to each 500 c. c. of nutrient solution.

Oat plants (Foundation Wisconsin Wonder Stock Pedigree No. 1) were used in all experiments. These were germinated in moistened pure quartz sand, and the seedlings were supported over the nutrient solution by the method described by Tottingham (*15*). When arsenic trioxide was used in the solution, it was added at the rate of a definite number of parts per million calculated as metallic arsenic.

The loss of weight by transpiration was determined at the end of each week by weighing the cultures, and enough distilled water was added at each weighing to compensate for the loss by transpiration. In some of the experiments the plants were severed just above the remains of the seeds, and the tops were dried at a constant temperature of 102° C. until they became constant in weight. The results of these experiments are recorded in Tables 1, 2, and 3.

TABLE 1.—*Loss of water by oat plants grown in Shive's nutrient solution with and without the addition of arsenic trioxide*

Culture No.	Arsenic, parts per million	Water lost (grams)				Appearance of plants
		First week	Second week	Third week	Total	
1	0	9.5	23.5	35.5	68.5	Normal color, leaf blade broad.
2	0	10.0	23.0	36.5	69.5	
3	1	10.0	19.0	20.5	49.5	Light-green color, leaf blades narrower than Nos. 1 and 2.
4	1	9.0	17.5	19.0	45.5	
5	3	6.5	12.0	14.0	32.5	Lighter colored, and leaf blades narrower than Nos. 3 and 4.
6	3	6.0	[a] 9.5	10.5	[b] 26	

[a] One plant made no growth, probably because of fungous attack.
[b] No allowance is made for dwarfed plant.

TABLE 2.—*Loss of water by oat plants grown in Shive's nutrient solution, with and without the addition of arsenic trioxide*

Culture No.	Arsenic, parts per million	Water lost (grams)					Dry weight of tops in grams	Appearance of plants
		First week	Second week	Third week	Fourth week	Total		
1	0	3.5	10.0	22.5	48.5	84.5	0.1990	Leaf blades broad, normal color, average height 400 mm. Average length roots 85 mm.
2	0	1.5	8.5	23.5	44.5	78.0	.1820	
3	0	3.0	10.0	24.5	50.0	87.5	.1974	
4	5	2.0	[a] 6.0	6.0	9.5	[b] 23.5	[b] .0630	Leaf blades slightly rolled, light-green color, average height 200 mm. Average length roots 25 mm.
5	5	2.5	4.5	4.5	12.0	23.5	.0622	
6	5	1.0	4.0	7.0	11.5	23.5	.0854	
7	10.	1.5	3.5	5.0	13.0	23.0	.0778	Color light green. Average height 165 mm. Average length roots 25 mm.
8	10	1.5	[a] 3.0	4.5	9.0	[b] 18.0	[b] .0591	
9	10	1.0	2.5	4.5	11.5	19.5	.0614	

[a] One plant dead.
[b] No allowance is made for dead plant.

TABLE 3.—*Loss of water by oat plants grown in Shive's nutrient solution, with and without the addition of arsenic trioxide*

Culture No.	Arsenic, parts per million	Water lost (grams)				Dry weight tops (grams)	Appearance of plants
		First week	Second week	Third week	Total		
1	0	12.0	31.0	56.0	99.0	0.3270	Good color, root system well developed; leaf blades broad.
2	0	8.5	30.5	58.5	97.5	.3160	
3	0	10.0	29.5	55.0	94.5	.3300	
4	5	7.0	17.0	13.5	37.5	.1212	Light-green color; root system poorly developed; leaf blades narrow.
5	5	6.5	13.5	12.0	32.0	.1090	
6	5	9.5	15.5	17.0	42.0	.0936	
7	10	5.0	8.0	8.5	21.5	.0682	Similar to 4, 5, and 6, but all effects more noticeable.
8	10	4.5	5.0	2.5	12.0	.0628	
9	10	8.5	9.0	9.5	27.0	.0694	

Tables 1, 2, and 3 show rather conclusively that arsenic added as arsenic trioxide decreases transpiration, even when added at the rate of one part per million. The treated plants were characterized by a lighter-green foliage and narrower leaf blades than the control. While all plants were treated similarly, the results indicate considerable individuality in the plants themselves.

TRANSPIRATION STUDIES WITH TOMATO PLANTS POTTED IN SOIL

Tomato plants were given the ordinary cultural care of a well-managed greenhouse until the plants were about 8 inches tall and of a size suitable for transpiration work. Throughout the experiments all plants were kept as nearly as possible under similar conditions of light, temperature, and humidity.

In determining the amount of water lost by transpiration the soil and pot were so sealed as to allow no loss of water except through the aerial parts. A quantity of water equal to that lost by the plants was added daily, all losses being determined by weighing on scales sensitive to 0.5 gm.

In beginning an experiment, transpiration records were taken for a period of days, usually six, and the plants were then divided into groups of three each. One of these groups was retained as a control, and to the others a single application of arsenic trioxide in solution was added in different proportions after weighing the plants on the sixth day, and the experiments continued. The arsenic trioxide was added as a definite number of parts per million of metallic arsenic based upon the amount of moisture in the soil. The results of these experiments and of similar experiments with sodium arsenite and potassium arsenite are shown in Tables 4 to 7 and in Figures 1 to 4.

TABLE 4.—*Daily loss of water by Everbearing tomato plants growing in soil, with and without the addition of arsenic trioxide, the chemical added after the plants were weighed on the sixth day*

SERIES 1

Day	Loss of water in grams by control				Loss of water in grams after addition of—											
					Arsenic 25 parts per million				Arsenic 37.5 parts per million				Arsenic 50 parts per million			
	a	b	c	Total	a	b	c	Total	a	b	c	Total	a	b	c	Total
1	41	39	29	109	34	29	44	107	33	31	44	108	35	26	51	112
2	31	29	23	83	24	20	31	75	23	25	29	77	24	20	32	76
3	60	63	48	171	44	41	79	164	51	46	63	160	48	40	63	151
4	48	45	33	126	41	34	54	129	37	38	48	123	47	36	49	132
5	72	66	49	187	51	46	83	180	63	53	71	187	59	49	71	179
6	67	54	37	158	53	41	66	160	49	44	62	155	64	50	55	169
7	68	76	54	198	46	50	82	178	62	46	71	179	53	44	55	152
8	61	56	44	161	45	43	58	146	44	38	50	132	53	39	46	138
9	88	91	72	251	68	63	102	233	65	59	77	201	83	66	68	217
10	106	100	72	278	71	71	103	245	80	69	93	242	87	72	81	240
11	112	93	70	275	65	70	101	236	90	61	88	239	77	68	76	221
12	80	63	49	192	45	47	68	160	58	44	55	157	58	48	53	159

SERIES 2

1	45	22	39	106	29	42	49	120	33	31	47	111	31	39	39	109
2	134	52	96	282	67	81	122	270	67	86	118	271	79	100	100	279
3	104	51	88	243	64	71	95	230	65	74	96	235	71	76	91	238
4	78	38	64	180	43	54	72	169	51	52	73	176	50	52	65	167
5	67	25	54	146	32	44	62	138	43	49	61	153	45	41	58	144
6	102	55	88	245	59	80	88	227	73	83	101	257	63	60	77	200
7	91	34	66	191	45	53	58	156	44	46	61	151	53	48	53	154
8	71	31	48	150	32	41	60	133	37	40	54	131	37	35	46	118
9	101	44	83	228	47	60	66	173	58	57	63	178	54	57	58	169
10	82	45	87	214	49	66	71	186	58	60	67	185	47	52	56	155
11	103	52	98	253	57	73	85	215	65	72	80	217	60	60	74	194
12	159	67	134	360	93	100	113	306	100	98	106	304	94	99	101	294

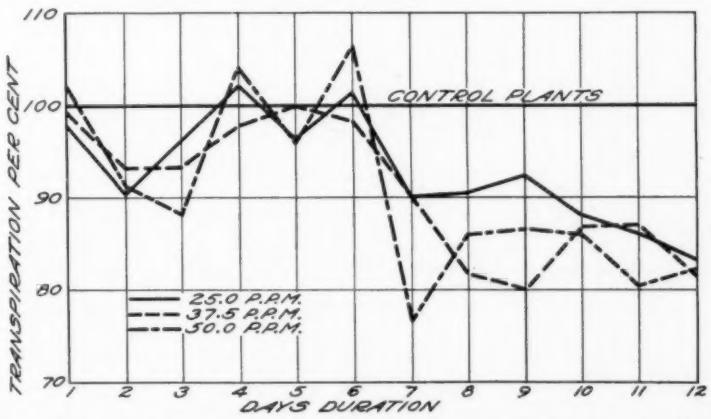


FIG. 1.—Daily loss of water by Everbearing tomato plants growing in soil, with and without the addition of 25, 37.5, and 50 parts per million of arsenic trioxide on the sixth day of the experiment. One hundred indicates total loss of water by the control plants. (Data from Table 4, series 1)

TABLE 5.—*Daily loss of water by Everbearing tomato plants growing in soil, with and without the addition of arsenic trioxide, the chemical added after the plants were weighed on the sixth day*

SERIES 1

Day	Loss of water in grams by control				Loss of water in grams after addition of—											
					Arsenic 10 parts per million				Arsenic 15 parts per million				Arsenic 20 parts per million			
	a	b	c	Total	a	b	c	Total	a	b	c	Total	a	b	c	Total
1	67	58	60	185	90	81	72	252	70	62	87	219	93	74	62	229
2	90	73	79	242	115	108	96	319	89	82	109	280	120	98	80	298
3	60	47	42	149	70	59	56	185	63	52	66	181	77	55	54	186
4	110	97	95	302	137	133	110	380	124	108	136	368	156	124	100	380
5	78	66	74	218	91	91	87	269	87	63	108	258	106	92	84	282
6	95	89	81	265	117	110	93	320	117	102	126	345	140	104	92	336
7	75	62	65	202	86	92	80	258	85	71	94	250	106	90	81	277
8	118	110	95	323	130	128	115	373	132	101	142	375	141	128	105	374
9	118	110	104	332	121	113	126	360	130	97	137	364	135	121	118	374
10	145	134	119	398	145	125	147	417	151	102	157	410	137	134	137	408
11	123	118	116	357	124	104	112	340	131	90	135	356	122	108	113	343
12	75	64	58	197	76	73	65	214	76	55	86	217	77	72	68	217

SERIES 2

1	244	166	188	598	170	176	133	479	203	219	204	626	161	193	214	568
2	199	142	153	494	149	145	125	419	156	183	167	506	146	159	193	498
3	252	194	194	640	190	187	165	542	176	220	228	624	196	205	242	643
4	190	154	154	498	145	172	149	466	148	195	176	519	159	165	179	503
5	268	217	221	706	213	229	203	645	205	237	244	686	241	231	266	738
6	173	135	140	448	133	164	146	443	134	164	151	449	148	142	159	449
7	150	114	117	381	105	134	133	372	111	130	126	367	125	121	137	383
8	116	87	94	297	84	107	92	283	88	101	100	289	94	92	98	284
9	180	131	132	443	133	151	136	420	135	141	143	419	146	139	152	437
10	153	122	128	403	126	161	138	425	125	138	147	410	130	135	145	410
11	148	113	120	381	129	142	121	392	119	126	131	376	131	124	140	395
12	97	80	88	265	91	111	90	292	81	100	102	283	92	91	97	280

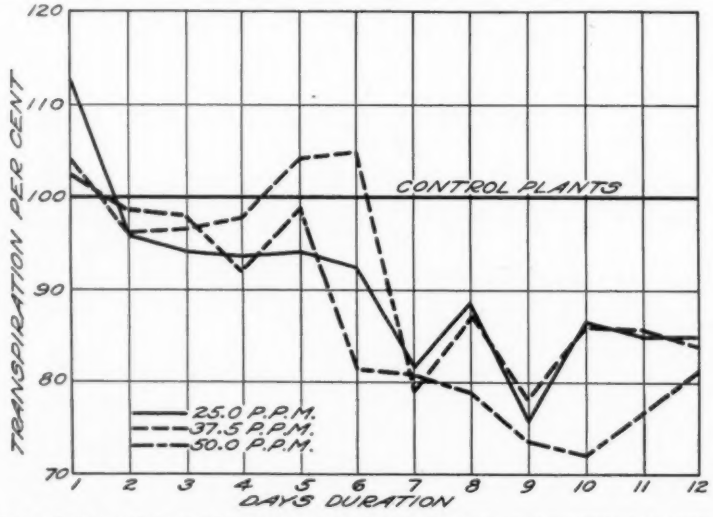


FIG. 2.—Daily loss of water by Everbearing tomato plants growing in soil, with and without the addition of 25, 37.5, and 50 parts per million of arsenic trioxide on the sixth day of the experiment. One hundred indicates total loss of water by the control plants. (Data from Table 4, series 2.)

TABLE 6.—*Daily loss of water by Earliana tomato plants growing in 6-inch pots of garden soil, with and without the addition of sodium arsenite (0.01 gm.) and potassium arsenite (0.10 gm.) the chemicals added after the plants were weighed on the sixth day*

Day	Loss of water in grams by—		
	Plant 1, untreated	Plant 2, treated with sodium arsenite	Plant 3, treated with potassium arsenite
1	115.0	142.5	129.0
2	113.0	135.5	111.0
3	83.5	102.0	83.5
4	66.0	85.0	72.5
5	121.0	156.5	150.0
6	201.0	271.5	246.0
7	194.5	217.0	153.5
8	231.5	296.5	157.0
9	141.0	150.5	82.5
10	245.0	287.0	152.0
11	283.5	277.5	138.5
12	165.0	188.5	107.0
13	201.5	232.0	122.0
14	182.5	222.0	111.5
15	265.0	270.5	127.0
16	250.5	242.5	125.0
17	210.5	216.5	131.0
18	167.5	178.5	96.0
19	104.5	113.0	59.0
20	242.5	245.5	139.0

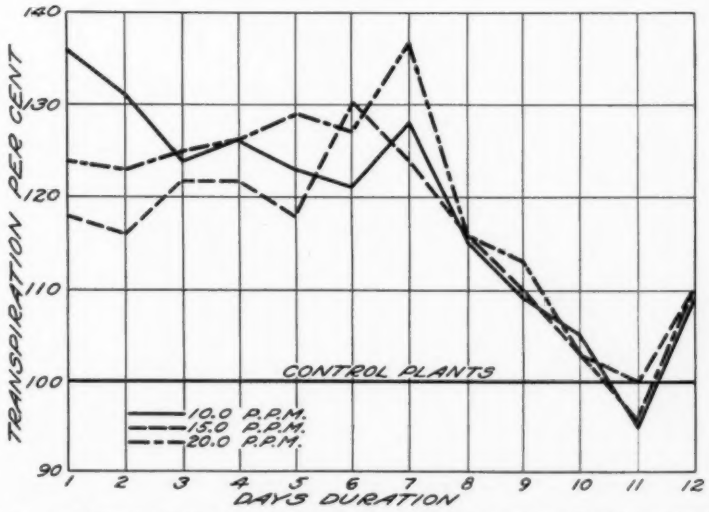


FIG. 3.—Daily loss of water by Everbearing tomato plants growing in soil, with and without the addition of 10, 15, and 20 parts per million of arsenic trioxide on the sixth day of the experiment. One hundred indicates total loss of water by the control plants. (Data from Table 5, series 1.)

TABLE 7.—*Daily loss of water by Earliana tomato plants growing in 6-inch pots of garden soil. Sodium arsenite (0.05 gm.) added to each pot after the plants were weighed on the sixth day*

TREATED PLANTS

Date	Loss of water in grams by plant No.						Total loss of water	Hours between weighing	Average loss (grams per hour)
	3	5	7	8	10	11			
1913									
Feb. 27	86.0	82.5	89.5	85.5	48.5	95.0	487.0	23.0	21.2
Feb. 28	78.5	85.5	98.0	99.0	50.5	103.5	515.0	25.0	20.6
Mar. 1	86.0	110.5	123.5	145.0	75.0	127.5	667.5	25.0	26.7
Mar. 2	80.0	62.0	61.5	76.5	39.0	68.0	387.0	22.0	17.6
Mar. 3	127.0	132.0	141.0	155.5	89.5	149.0	794.0	24.0	33.1
Mar. 4	95.5	101.5	95.0	128.0	64.0	112.0	596.0	24.0	24.8
Mar. 5	126.0	129.5	131.0	155.5	94.5	163.0	799.5	25.0	32.0
Mar. 6	60.5	63.0	71.0	86.0	45.0	82.0	407.5	23.0	17.7
Mar. 7	67.0	94.5	102.0	113.5	82.0	142.0	601.0	24.0	25.0
Mar. 8	90.0	113.0	118.0	108.0	82.0	117.0	628.0	24.0	26.2
Mar. 9	111.0	133.0	138.0	145.0	98.0	225.0	850.0	25.0	34.0
Mar. 10	129.0	166.0	145.0	165.0	110.0	200.0	915.0	27.5	33.3
Mar. 11	37.0	47.0	37.0	43.0	28.0	47.0	239.0	19.3	12.4
Mar. 12	77.0	108.0	98.0	96.0	77.0	112.0	568.0	24.0	23.7
Mar. 13	84.0	118.0	93.0	92.0	90.0	113.0	590.0	24.0	24.6
Mar. 14	70.0	100.0	83.0	73.0	92.0	95.0	513.0	24.0	21.4
Mar. 15	113.0	169.0	130.0	114.0	159.0	178.0	863.0	25.0	36.5
Mar. 16	112.0	150.0	120.0	107.0	119.0	152.0	760.0	24.5	31.0
Mar. 17	85.0	115.0	95.0	85.0	113.0	114.0	607.0	23.0	26.4
Mar. 18	66.0	111.0	82.0	82.0	131.0	140.0	612.0	24.5	25.0
Mar. 19	146.0	211.0	147.0	141.0	124.0	124.0	893.0	29.5	30.3
Mar. 20	83.0	48.0	80.0	77.0	102.0	87.0	477.0	24.5	19.5
Mar. 21	60.0	103.0	69.0	52.0	90.0	85.0	459.0	22.5	20.4
Mar. 22	104.0	157.0	109.0	82.0	124.0	107.0	683.0	24.0	28.5
Mar. 23	54.0	93.0	68.0	43.0	57.0	65.0	380.0	21.0	18.1
Mar. 24	116.0	221.0	160.0	109.0	149.0	180.0	935.0	29.0	32.2
Mar. 25	84.0	122.0	87.0	57.0	83.0	90.0	523.0	24.0	21.8
Mar. 26	78.0	117.0	86.0	64.0	98.0	88.0	531.0	23.0	23.1
	2,505.5	3,263.0	2,857.5	2,779.5	2,514.0	3,361.0	17,280.5		

CONTROLS

Date	Loss of water in grams by plant No.						Total loss of water	Hours between weighing	Average loss (grams per hour)
	1	2	4	6	9	12			
1913									
Feb. 27	79.5	94.0	96.0	102.5	85.0	69.0	526.0	23.0	22.9
Feb. 28	101.5	102.0	84.0	92.0	89.0	65.0	533.5	25.0	21.3
Mar. 1	105.0	100.5	118.5	112.5	109.0	81.5	627.0	25.0	25.1
Mar. 2	96.0	96.5	60.5	58.0	56.0	41.0	408.0	22.0	18.5
Mar. 3	152.0	154.0	138.5	130.0	123.0	68.0	765.5	24.0	31.9
Mar. 4	124.0	119.0	92.0	90.5	81.5	57.5	564.5	24.0	23.5
Mar. 5	145.5	151.0	140.0	132.0	134.0	108.0	810.5	25.0	32.4
Mar. 6	74.0	80.0	55.5	71.0	65.0	56.0	401.5	23.0	17.5
Mar. 7	156.0	167.0	151.0	149.0	161.0	134.0	918.0	24.0	38.3
Mar. 8	176.0	204.0	157.0	181.0	160.0	117.0	995.0	24.0	41.5
Mar. 9	178.0	195.0	163.0	167.0	210.0	143.0	1,056.0	25.0	42.2
Mar. 10	197.0	225.0	200.0	170.0	207.0	137.0	1,136.0	27.5	41.3
Mar. 11	77.5	81.0	73.0	47.0	56.0	43.0	377.5	19.3	19.6
Mar. 12	129.5	130.5	139.5	102.0	144.0	102.0	747.5	24.0	31.1
Mar. 13	131.0	142.0	150.0	109.0	172.0	122.0	826.0	24.0	34.4
Mar. 14	112.0	114.0	128.0	97.0	140.0	103.0	694.0	24.0	28.9
Mar. 15	176.0	219.0	208.0	169.0	239.0	183.0	1,194.0	25.0	47.8
Mar. 16	179.0	201.0	173.0	139.0	162.0	122.0	976.0	24.5	39.8
Mar. 17	131.0	143.0	159.0	105.0	152.0	117.0	807.0	23.0	35.1
Mar. 18	100.0	111.0	107.0	78.0	199.0	148.0	743.0	24.5	30.3
Mar. 19	206.0	229.0	217.0	170.0	184.0	139.0	1,145.0	29.5	38.8
Mar. 20	109.0	137.0	126.0	117.0	164.0	146.0	799.0	24.5	32.6
Mar. 21	127.0	146.0	158.0	116.0	176.0	154.0	877.0	22.5	39.0
Mar. 22	213.0	234.0	219.0	175.0	219.0	180.0	1,240.0	24.0	51.7
Mar. 23	106.0	124.0	82.0	90.0	99.0	90.0	591.0	21.0	28.1
Mar. 24	271.0	316.0	246.0	230.0	264.0	211.0	1,538.0	29.0	53.0
Mar. 25	148.0	162.0	143.0	117.0	169.0	117.0	856.0	24.0	35.7
Mar. 26	142.0	151.0	168.0	123.0	175.0	169.0	928.0	23.0	40.3
Mar. 27	273.0	309.0	249.0	214.0	246.0	209.0	1,500.0	25.0	60.0
Mar. 28	153.0	147.0	145.0	98.0	201.0	109.0	853.0	23.0	37.1
Mar. 29	116.0	85.0	99.0	57.0	73.0	43.0	473.0	24.0	19.7
Mar. 30	144.0	80.0	105.0	64.0	58.0	39.0	499.0	24.0	20.8
Mar. 31	119.0	77.0	88.0	53.0	44.0	30.0	411.0	25.0	16.4
Apr. 1	81.0	63.0	72.0	42.0	41.0	28.0	327.0	23.0	14.2
Apr. 2	173.0	118.0	138.0	84.0	74.0	65.0	652.0	26.0	25.1
	5,001.5	5,216.5	4,848.5	4,051.5	4,931.5	3,746.0	27,795.5		

From a study of these tables and figures it is apparent that arsenic added as arsenic trioxide decreased the transpiration of tomato plants to a greater or less extent when the plants were grown in soil. The tables emphasize the fact that considerable variation in the quantity of water lost by the same plant occurs from day to day. These variations are due mainly to meteorological conditions.

In Figure 1 it is shown that a decided effect was produced on transpiration by the addition of arsenic trioxide in quantities of 25, 37.5, and 50 parts per million. While some variation occurred from day to day, at the end of the experiment the loss of water by the treated plants was from 80 to 85 per cent of that lost by the controls as compared to 97 per cent or more at the time the chemical was added. Figure 2, a graphic representation of a duplicate of this experiment, shows similar results.

Figures 3 and 4, where smaller quantities of arsenic trioxide were used, indicate a considerable effect on transpiration from the addition

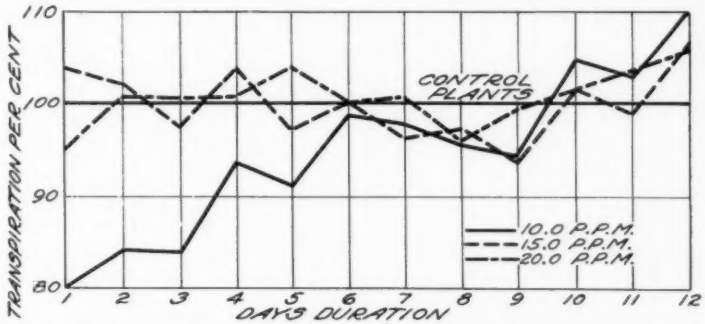


FIG. 4.—Daily loss of water by Everbearing tomato plants growing in soil, with and without the addition of 10, 15, and 20 parts per million of arsenic trioxide on the sixth day of the experiment. One hundred indicates total loss of water by the control plants. (Data from Table 5, series 2.)

of the chemical. In Figure 3 the treated plants on the sixth day averaged 126 per cent total transpiration in comparison with that of the controls, and on the eleventh day they averaged only 97 per cent, indicating that the chemical influenced transpiration.

Figure 4 shows a small decrease for three days, and then a marked tendency toward recovery. In all cases where only small quantities of arsenic trioxide were added, the plants showed a tendency to recover after a certain length of time, which in these experiments varied usually from 2 to 5 days.

The fact that the loss of water by the control plants shown in the different graphs was higher or lower than by the treated plants is not significant since there was considerable variation and all data were comparable.

TRANSPIRATION STUDIES WITH TOMATO PLANTS POTTED IN SAND

Quartz sand No. 3.5, a commercial product obtained from Ottawa, Ill., was screened through standard 20 and 40 mesh sieves, and only that part was used which passed through the 20-mesh sieve and was retained by the 40-mesh. The sand was thoroughly digested with

weak hydrochloric acid for 48 hours and then washed with distilled water until no acid reaction was noted with methyl orange as in indicator. The water-retaining capacity of the sand was about 11 per cent.

The tomato plants used in the experiments were grown in soil, and at the time of transplanting were about 5 inches high. As much of the soil as possible was removed from their roots and they were transplanted in glazed earthenware jars containing 2,500 gm. of air-dried sand. The nutrient solution (p. 3) was added to bring the moisture content to 15 per cent of the air-dried weight of the sand.

The plants were allowed to grow for about a month before the experiment was begun. During this interval an additional 125 gm. of the nutrient solution was added besides the distilled water necessary to replace the loss through transpiration.

During the experiment the jars were so sealed as to allow no loss of water except through the aerial parts of the plant. Daily an amount of distilled water equal to that lost by the plant was added. The loss was determined by weighing on scales sensitive to 0.5 gm. Arsenic trioxide in solution was added to the plants by a method similar to that described under soil cultures. The results of this experiment are shown in Table 8 and Figure 5.

TABLE 8.—*Daily loss of water by Everbearing tomato plants growing in sand, with and without the addition of arsenic trioxide, the chemical added after the plants were weighed on the sixth day*

Day	Loss of water by control				Loss of water after addition of arsenic, 10 parts per million				Loss of water after addition of arsenic, 20 parts per million			
	a	b	c	Total	a	b	c	Total	a	b	c	Total
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	60	35	60	155	22	32	39	93	46	47	45	138
2	90	55	98	243	30	47	60	137	75	74	75	224
3	65	40	70	175	22	32	44	98	55	51	52	158
4	101	70	112	283	38	66	76	180	89	83	91	263
5	65	49	77	191	27	43	54	124	60	57	65	182
6	102	80	110	292	48	70	80	198	97	88	109	294
7	63	52	69	184	25	30	30	85	25	33	33	91
8	71	67	90	228	19	24	26	69	21	24	20	65
9	82	81	107	270	13	27	33	73	18	22	22	62
10	106	105	142	353	23	39	46	108	20	29	27	76
11	54	45	59	158	16	20	26	62	14	19	19	52
12	43	35	57	135	16	23	28	67	16	25	23	64

From these it is apparent that arsenic decreases the transpiration of tomato plants when they are grown in sand. Table 8 shows that considerable variation in the loss of water occurs from day to day, a fact which is mainly due to meteorological conditions. The injury is apparent in a shorter period of time in sand than in soil, when equal quantities of arsenic trioxide are added and transpiration is used as a criterion. Compare Figure 3 with Figure 5. After the fourth day there is a marked tendency toward recovery.

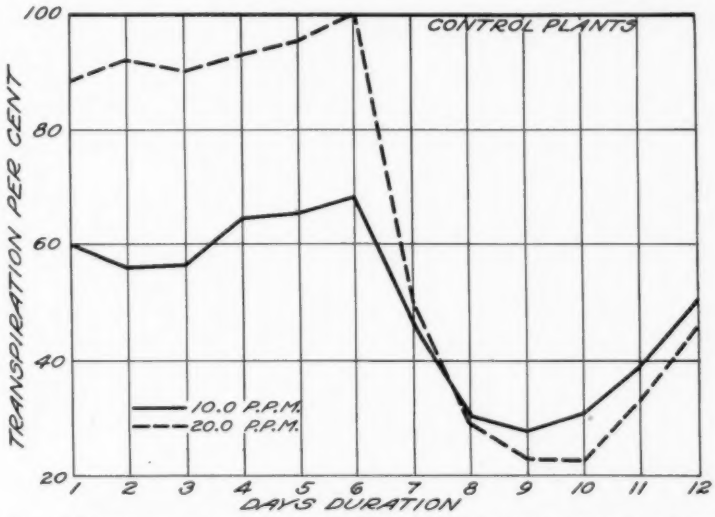


FIG. 5.—Daily loss of water by Everbearing tomato plants growing in sand, with and without the addition of 10 and 20 parts per million of arsenic trioxide on the sixth day of the experiment. One hundred indicates total loss of water by the control plants. (Data from Table 8.)

EFFECT ON GROWTH OF POTTED PLANTS CAUSED BY ADDITION OF SOLUBLE ARSENICAL COMPOUNDS TO THE SOIL

The plants in this group of experiments were grown in 6-inch pots full of garden soil. Table 9 gives the variety of plant and its condition at the time of treatment, and Table 10 shows the effect produced by the chemicals on the various plants. Potassium or sodium arsenite proved to be highly toxic to practically all the plants. The other compounds were only slightly toxic to the cereals. In the same family or group there was noted a wide variation in tolerance of the chemical, as, for example, arsenic acid and potassium arsenate in the quantities added proved fatal to radish and caused only slight injury to turnip. Sodium arsenate killed the oat plants, but apparently caused no injury to barley or wheat.

TABLE 9.—*Condition of plants prior to treatment with soluble arsenicals as shown in Table 10*

Plant	Variety	Height in inches	Condition
Barley	New Zealand	10–14	Stems slender, normal color.
Bean A	Prolific German Wax	15–25	Vigorous growth, normal color.
Bean B	White Navy	15–25	Vigorous growth, normal color.
Cabbage	Large Premium	6–12	Fair condition, light green in color.
Clover	Mammoth Red	5	Leaves normal in size and color.
Corn	White Flint	21–27	Stalks slender, normal color.
Cucumber	Improved Long Green	8–12	Vigorous growth, normal color.
Lettuce	Hanson	6	Leaves 4–5 inches wide, normal color.
Oats	Swedish Select White Wonder	10–18	Stems weak but erect, normal color.
Pea	Canadian Field	7–22	Somewhat spindling growth, normal color.
Radish	White Strasburg	8–14	6–8 leaves, stems slender, normal color.
Squash	Hubbard	8–12	Vigorous growth, normal color.
Timothy		12–20	Spindling growth of stems, light green color.
Tomato A	Early Michigan	8–10	Vigorous growth, normal color.
Tomato B	Perfection	8–10	Vigorous growth, normal color.
Turnip		6–12	Roots small to 2 inches in diameter, normal color.
Wheat	Glyndon 650	14–20	Stems somewhat weak, normal color.

TABLE 10.—*Effect on potted plants caused by the addition to the soil of 0.5 gm. of soluble arsenicals*

Plant used	Effect on plant caused by addition to soil of—					
	Arsenic acid	Ammonium arsenate	Potassium arsenate	Sodium arsenate	Potassium arsenite	Sodium arsenite
Barley	No injury	No injury	No injury	No injury	Very bad	Dead.
Bean A	Dead	Very bad	Dead	Dead	Dead	Do.
Bean B	"	Dead	do	do	do	Do.
Cabbage	"	"	"	"	"	Do.
Clover	"	"	"	Dead	"	Do.
Corn	No injury	No injury	Moderate	No injury	Very bad to dead	Do.
Cucumber	Dead	Dead	"	"	"	Do.
Lettuce	do	"	No injury	No injury	Dead	Do.
Oats	No injury	No injury	do	Dead	Bad	Do.
Pea	"	"	"	"	"	Do.
Radish	Dead	No injury	Dead	No injury	Dead	Do.
Squash	"	"	"	do	"	Do.
Timothy	"	"	"	"	"	Do.
Tomato A	Dead	Dead	Moderate	No injury	Dead	Do.
Tomato B	Bad	do	No injury	do	do	Do.
Turnip	No injury	No injury	Slight	do	No injury	Bad.
Wheat	do	do	No injury	do	Dead	Dead.

EFFECT ON GROWTH OF POTTED PLANTS CAUSED BY ADDITION OF INSOLUBLE ARSENICAL COMPOUNDS TO THE SOIL

The plants in these experiments were grown in 6-inch pots full of garden soil. Table 11 shows the effect on growth produced by adding 2.5 gm. of the arsenical, which was sprinkled as evenly as possible over the surface of each pot. The control plants made a satisfactory growth, and remained in a good healthy condition throughout the experiment.

TABLE 11.—*Effect on the growth of various plants in 6-inch pots caused by the addition to the soil of 2.5 gm. of arsenic compounds*

Arsenic compound used	Effect of arsenical on—					
	Barley	Carrot	Oats	Sugar beet	Tomato A	Wheat
Arsenic (metal)	Very injurious.	Dead				
Arsenic trioxide	No injury	Slightly stunted			Slightly stunted.	
Calcium arsenate	do	Moderately stunted			do	
Copper arsenate		Badly stunted	No injury		No injury	
Ferrous arsenate		do			do	
Lead arsenate		Slightly stunted	No injury		do	
Mercury(ic) arsenate		do				No injury.
Zinc arsenate		Badly stunted		Dead	Dead	Do.
Calcium arsenite				do		Dead.
Copper arsenite			No injury	Slightly stunted.	Dead	
Iron and ammonia arsenite		Moderate stunted		No injury		Dead.
Zinc arsenite		do		do		
Arsenic sulphide, red				do		
Lead arsenite			No injury		Injurious	

The so-called "insoluble" arsenical compounds are arranged below in the order of their toxicity as indicated by the injury which they caused to bean and tomato. Those listed first caused severe injury; those preceded by an asterisk caused none.

BEAN

Amonium arsenate	Lead arsenite	Lead arsenate
Zinc arsenate	Copper arsenite	Mercuric arsenate
Arsenic metal	Zinc arsenite	Ferric arsenite and ammonium citrate
Calcium arsenite	Arsenic trioxide	*Arsenic disulphide, red
Mercurous arsenite	Calcium arsenate	*Copper arsenate
Arsenic sulphide, yellow	Ferrous arsenate	

TOMATO

Arsenic metal	Arsenic sulphide, yellow	*Ferrous arsenate
Calcium arsenite	Zinc arsenite	*Lead arsenite
Ammonium arsenate	Arsenic trioxide	*Ferric arsenite and ammonium citrate
Mercurous arsenite	Mercuric arsenate	*Arsenic disulphide, red
Copper arsenite	*Calcium arsenate	*Lead arsenate
Zinc arsenate	*Copper arsenate	

These results indicate that plants show an individuality in their reaction to chemical compounds, which may vary greatly with different species.

EFFECT ON GROWTH OF CROPS CAUSED BY INCORPORATION OF ARSENICAL COMPOUNDS IN THE SOIL

The ground selected for the work reported in this section was a uniform strip of medium clay loam slightly sloping to the west. The ground was prepared by the ordinary method used for making a good seed bed. It was then staked out into plots 3 feet square with 2-foot alleys running north and south and east and west. Figure 6 shows the arrangement of the plots, the cropping system, and the arsenical compounds used. This system remained uniform throughout the experiment unless otherwise noted. Each year the plots except the alfalfa, clover, and timothy were spaded, thoroughly worked, and planted. The original planting was as follows: Wheat, timothy, alfalfa, and clover in 5 rows running north and south, and 6 inches apart. Sugar beets and field peas in 3 rows running north and south, and 12 inches apart. The first row in all cases was 6 inches from the margin of the plot. Potatoes, cabbage, and tomatoes were set 4 plants to a plot, equidistant and 10 inches from the margin.[3] Cucumbers were planted about 2 inches apart in a circular hill 16 inches in diameter.

The plots were watered by a revolving sprinkler to prevent any washing of the surface soil. The chemicals used and the quantities added at each treatment were as follows (each plot received an equivalent amount of arsenic, approximately 11 gm., as determined by the results of analysis made by members of the chemistry department of the Montana station):

Chemical	Grams per treatment	Chemical	Grams per treatment
Arsenic trioxide	14. 58	Lead arsenate ertho	63. 10
Arsenic trisulphide	18. 03	Lead arsenite	30. 50
Calcium arsenite	20. 82	Sodium arsenite	18. 33
Copper aceto-arsenite	24. 55	Sodium arsenate	43. 07
Lead arsenate pyro	50. 00	Zinc arsenite	20. 10

[3] The cabbage and tomato plants were transplanted from 4-inch pots when they were about 5 inches tall

TABLE 12.—*Growth of plants in plots of soil treated with different arsenicals*

CABBAGE

Year	Plot treated with—											
	Arsenic trioxide	Arsenic trisulphide	Calcium arsenite	Copper aceto-arsenite	Lead arsenate acid	Lead arsenate ortho	Lead arsenite	Sodium arsenate	Sodium arsenite	Zinc arsenite	Control 1	Control 2
1911 [a]	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good.
1912	Fair	Moderate	Fair	Poor	Moderate	Poor	Fair	Fair	Moderate	Poor	do	Do.
1913 [b]	do	Poor	None	do	do	Good	Poor	None	Poor	None	do	Do.
1914 [c]												
1915 [b]	Moderate	Fair	Poor	do	Good	Moderate	None	Poor	None	Fair	do	Moderate.
1916	Good	do	do	do	do	Good	Good	None	Moderate	do	do	Good.
1917 [a]	Fair	Poor	do	do	Moderate	Moderate	Fair	Fair	Fair	Poor	do	Do.
1918	Moderate	Fair	do	Fair	Good	Good	Moderate	Moderate	Moderate	do	do	Do.
1919	None	None	Moderate	Moderate	Fair	Moderate	Good	Fair	Good	Fair	do	Moderate.
1920	Poor	Moderate	Fair	Fair	Moderate	do	Fair	Moderate	do	Poor	do	Good.
1921	do	Fair	do	do	Good	do	Good	Fair	Fair	do	Moderate	Do.
1922	Good	Good	Good	Good	do	Good	do	Good	Good	Good	Good	Do.

CUCUMBER

1911 [a]	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good.
1912	Moderate	Fair	None	Poor	Moderate	Moderate	Poor	Poor	Fair	None	do	Do.
1913 [b]	None	None	do	None	None	None	None	None	None	do	do	Do.
1914	do	do	do	do	do	do	do	do	do	do	Moderate	Do.
1915 [a]	do	do	do	do	Poor	do	do	Poor	do	do	Good	Do.
1916	Poor	Poor	do	Poor	do	do	do	do	do	Poor	do	Do.
1917	None	do	do	None	do	Poor	do	do	Poor	do	do	Do.
1918	Poor	Fair	Poor	Fair	do	None	Poor	do	do	Fair	do	Do.
1919	do	Poor	do	Poor	do	do	do	Fair	Fair	Poor	do	Do.
1920	do	do	do	do	Fair	do	do	do	do	do	do	Do.
1921	do	Fair	do	do	Poor	Poor	do	do	do	Fair	do	Do.
1922 [d]												

FIELD PEA

1911 [a]	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good.
1912	do	Moderate	Moderate	Moderate	do	do	Moderate	Moderate	Moderate	Poor	do	Do.
1913 [b]	do	Poor	None	Fair	do	do	Poor	Poor	Fair	None	do	Do.
1914	do	do	do	Poor	Moderate	Fair	do	do	Poor	do	do	Do.

1915 [b]	Moderate	Fair	Fair	Fair	do	Moderate	Fair	do	Fair	Fair	do	Do.
1916	Poor	None	None	None	Poor	Poor	None	None	None	None	do	Do.
1917	do	Poor	do	Poor	Fair	Fair	Poor	do	Poor	Poor	do	Do.
1918	do	Moderate	Fair	Moderate	Moderate	do	Fair	Fair	Fair	Fair	do	Do.
1919	Good	Fair	Poor	do	do	do	do	Moderate	Moderate	Good	do	Do.
1920	Moderate	do	do	Fair	Good	Moderate	Poor	Good	Fair	Poor	do	Do.
1921	Fair	Moderate	Moderate	Poor	None	Poor	do	Fair	None	Moderate	do	Do.
1922	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	do	Do.

POTATO

1911 [a]	Good	Moderate	Poor	Fair	Good	Moderate	Fair	Moderate	Poor	Fair	Good	Good.
1912	do	Good	Good	Good	do	Good	Good	Good	Good	Good	do	Do.
1913 [b]	do	do	do	do	do	do	do	Moderate	do	do	do	Do.
1914	do	Fair	Fair	Poor	Moderate	Moderate	Fair	Poor	Fair	Poor	do	Fair.
1915 [b]	Moderate	do	Poor	Fair	Good	Good	do	do	do	do	do	Moderate.
1916	Good	Moderate	Fair	do	do	do	Moderate	do	do	do	do	Do.
1917 [a]	do	Fair	do	do	Moderate	Moderate	Fair	Fair	Moderate	do	do	Good.
1918	do	do	Good	Good	Good	Good	Moderate	do	Fair	do	Moderate	Do.
1919	Moderate	Poor	Fair	Fair	do	Moderate	Good	Good	do	do	do	Do.
1920	Fair	Moderate	Moderate	Moderate	do	Good	Moderate	do	Moderate	Fair	Fair	Do.
1921	Poor	Fair	Good	do	do	do	Good	do	do	do	do	Moderate.
1922	Good	Good	do	Good	do	do	do	do	Good	Good	Good	Good.

TOMATO

1911 [a]	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good.
1912	do	do	do	do	do	do	do	do	do	do	do	Do.
1913 [b]	Moderate	Moderate	Fair	Fair	Moderate	Moderate	Moderate	Moderate	do	Poor	do	Do.
1914	do	Fair	do	do	do	do	Fair	Fair	Moderate	do	do	Do.
1915 [b]	do	do	do	do	do	do	do	do	Fair	do	do	Do.
1916	Good	Moderate	Poor	Moderate	Good	Good	do	Poor	Poor	None	do	Do.
1917 [a]	Moderate	Poor	None	None	Moderate	Moderate	Poor	do	do	do	do	Do.
1918	Fair	do	Poor	Poor	Good	Good	Moderate	do	do	do	do	Do.
1919	Good	Moderate	do	Moderate	do	do	Fair	do	Moderate	do	do	Do.
1920	Moderate	Fair	do	do	do	do	Moderate	Moderate	Good	Fair	Moderate	Fair.
1921 [c]												
1922 [c]												

[a] Chemical treatment given that year.
[b] Treatment before planting and after harvest.
[c] Growth was generally very poor in 1914 on account of injury caused by worms.
[d] Plots not planted.
[e] No difference observed.

TABLE 12—*Growth of plants in plots of soil treated with different arsenicals*—Continued

SUGAR BEET

Year	Plot treated with—											
	Arsenic trioxide	Arsenic trisulphide	Calcium arsenite	Copper aceto-arsenite	Lead arsenate acid	Lead arsenate ortho	Lead arsenite	Sodium arsenate	Sodium arsenite	Zinc arsenite	Control 1	Control 2
1911 [a]	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good.
1912	do	Poor	Moderate	Moderate	Moderate	Moderate	Fair	Moderate	do	Fair	do	Do.
1913 [b]	Poor	Moderate	Fair	do	Fair	Fair	do	Poor	Fair	do	do	Do.
1914	Good	Good	Very poor	Poor	Good	Moderate	Moderate	do	do	Very poor	do	Do.
1915 [b]	do	Moderate	Fair	Moderate	do	Good	do	do	Moderate	None	do	Do.
1916	Fair	Poor	None	Poor	Fair	Moderate	Poor	None	None	do	do	Do.
1917 [a]	Poor	Very poor	do	None	Moderate	do	do	Poor	Poor	do	do	Do.
1918	Moderate	Fair	Good	Moderate	do	Fair	Moderate	do	Fair	Good	do	Do.
1919	Fair	Good	Fair	Fair	Good	Good	do	do	Poor	do	Moderate	Moderate.
1920	Moderate	Moderate	Poor	do	Fair	Fair	Fair	Moderate	Good	Moderate	Good	Fair.
1921	Fair	Good	Moderate	do	do	do	Moderate	Fair	Fair	do	do	Good.
1922	Moderate	Fair	do	do	Good	Moderate	Good	Moderate	Moderate	do	do	Do.

WHEAT

1911 [a]	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good.
1912	do	do	Moderate	Moderate	do	do	do	Fair	do	Fair	do	Do.
1913 [b]	do	do	Poor	Fair	do	do	Poor	None	Poor	None	do	Do.
1914	do	Poor	do	Poor	Moderate	do	do	do	None	do	do	Do.
1915 [b]	Moderate	Fair	Fair	Fair	do	Moderate	Fair	Poor	Poor	Fair	do	Do.
1916	do	Poor	Poor	Poor	Fair	Fair	Poor	None	None	Poor	do	Do.
1917 [a]	do	do	None	None	do	do	do	Poor	Poor	None	do	Do.
1918	None	None	Poor	Poor	Moderate	Moderate	None	do	do	do	do	Do.
1919	do	Poor	Fair	Fair	Good	do	Fair	Fair	Fair	Poor	do	Do.
1920	Poor	None	Poor	Poor	do	do	Poor	Moderate	None	None	do	Do.
1921	do	do	do	Fair	do	Good	Fair	do	Fair	do	do	Do.
1922	do	do	Fair	do	do	do	Poor	Good	Poor	do	Moderate	Do.

[a] Chemical treatment given that year.
[b] Treatment before planting and after harvest.

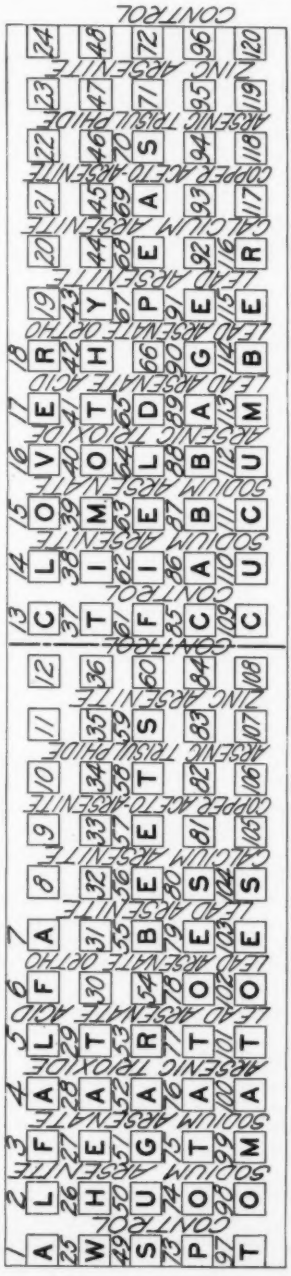


FIG. 6.—Diagram showing arrangement of plots, cropping system, and arsenical used in experiments made to determine the effect on the growth of crops caused by the incorporation of arsenical compounds in the soil

During the first year only the chemical was added by sprinkling it evenly over the surface of the soil while the crops were growing. All the other treatments were given in the fall after the crops were harvested, or in the spring before planting and incorporated in the soil.

The alfalfa, clover, and timothy plots proved very unsatisfactory owing to winter-killing and the intrusion of other grasses and weeds. These plots were abandoned and no detailed notes were taken on them.

Tables 12 and 13 indicate the time of treatment and the yearly rating of the season's growth of the different crops, namely, cabbage, cucumber, field peas, potatoes, tomato, sugar beet, and wheat, and the toxicity of the various arsenicals in relation to the crop.

TABLE 13.—*Toxicity of arsenicals to different crops* [a]

[Ranking from 1 (poor growth) to 10 (approximately normal growth)]

Arsenical	Toxicity of arsenical to—					
	Cabbage	Peas	Potato	Tomato	Sugar beet	Wheat
Arsenic trioxide	5	9	9	8	6	8
Arsenic trisulphide	6	5	3	4	9	7
Calcium arsenite	3	2	4	2	2	3
Copper aceto-arsenite	2	4	5	5	8	4
Lead arsenate acid	10	10	10	9	10	10
Lead arsenate ortho	9	8	8	10	7	9
Lead arsenite	8	1	7	6	3	6
Sodium arsenate	4	7	6	3	1	2
Sodium arsenite	7	3	2	7	4	5
Zinc arsenite	1	6	1	1	5	1

[a] All arsenicals injured the growth of cucumbers to such an extent that ranking was impossible.

From a critical examination of the data contained in Table 12 it appears that some chemicals become inactive very much sooner than others and that the amount of injury caused by the chemicals varies from year to year with different crops. In all the experiments the final addition of arsenic was made in 1917, and the plots were continually cropped until 1922 in order to determine the rapidity with which the soil would again produce normal growth. At no time did lead arsenate cause much injury, whereas zinc arsenite caused severe injury throughout the entire time. The degree of injury attributable to the other chemicals ranged between these extremes, as is shown by the data in Table 12.

SUMMARY

The results of transpiration studies with oats in water cultures show conclusively that arsenic added as arsenic trioxide decreases transpiration even when added at the rate of one part per million. The characteristics of treated plants are narrower leaf blades and a lighter color. There is apparent individuality in the reaction of plants to this arsenical.

Decreased transpiration resulted when arsenic trioxide was added at the rate of 10 parts per million or more to soil in which tomato plants were growing, and this decrease was more apparent in direct

proportion to the amount of arsenic added until serious injury or death resulted.

Similar results were obtained when sodium arsenite or potassium arsenite was substituted for arsenic trioxide.

When sand was used instead of soil, the other environmental conditions remaining the same, the injury was apparent in a shorter time. Considerable variation occurred from day to day, mainly due to meteorological conditions.

The addition of small quantities of soluble arsenical compounds to potted plants caused serious injury to most of the plants under test. As a rule the cereals were hardier than the other crops. Turnip was also fairly resistant. The arsenites were decidedly more toxic than the arsenates.

Some of the so-called "insoluble" arsenical compounds proved very toxic to plants when 2.5 gm. of the chemical was sprinkled evenly over the surface of the pot.

The toxicity of arsenical chemicals to plants varied. Some species showed a high, and to others a low, degree of tolerance to the same arsenical.

The incorporation of arsenical compounds in the soil is a dangerous practice, and may cause considerable injury as the concentration of arsenic increases. Arsenical compounds differ in their reaction in the soil, some becoming inert in a much shorter period than others. Plants also differ in their ability to withstand arsenic, as is illustrated by the fact that some crops remain approximately normal when arsenic in some form is present, while other crops in the same environment are killed. Beans and cucumbers are very susceptible to arsenic, but the cereals and grasses are much more resistant.

LITERATURE CITED

(1) Anonymous.
1859. [the presence of arsenic in plants cultivated for food.] Gard. Chron. 37: 739–740.

(2) Blyth, A. W.
1885. poisons; their effects and detection. 2 v., illus. New York.

(3) Candolle, A. P. de
1832. physiologie végétale. 3 v. Paris.

(4) Chatin, A.
1845. études de physiologie végétale faites au moyen de l'acide arsénieux. Compt. Rend. Acad. Sci. [Paris] 20: 21–29.

(5) Daubeny, C.
1862. on the power ascribed to the roots of plants of rejecting poisonous or abnormal substances presented to them. Jour. Chem. Soc. [London] 14: 209–230.

(6) Freytag, M.
1870. wissenschaftliches gutachten über den einfluss, welchen die hüttenwerke der mansfelder kupferschieferbauenden gewerkschaft auf die vegetation und indirect auf menschen und thiere ausüben. Eisleben. [Not seen.]

(7) Gorup-Besanez, E. von
1863. ueber das verhalten der vegetirenden pflanze und der ackererde gegen metallgifte. Ann. Chem. u. Pharm. (n. R. 51) 127: 243–256.

(8) Headden, W. P.
1908. arsenical poisoning of fruit trees. Colo. Agr. Expt. Sta. Bul. 131, 27 p., illus.

(9) Lyttkens, A.
1894. arseniks inflytande på växter. K. Landtbr. Akad. Handl. och Tidskr. 33: 317–320, illus.

(10) McMurtie, W.
1876. THE INFLUENCE OF ARSENICAL COMPOUNDS, WHEN PRESENT IN THE SOIL, UPON VEGETATION. [U. S.] Commr. Agr. Rpt. 1875: 144–147, illus.
(11) Nobbe, F., Baessler, P., and Will, H.
1884. UNTERSUCHUNG. ÜBER DIE GIFTWIRKUNG DES ARSEN, BLEI UND ZINK IM PFLANZLICHEN ORGANISMUS. Landw. Vers. Sta. 30: 381–423.
(12) Phillips, F. C.
1882. THE ABSORPTION OF METALLIC OXIDES BY PLANTS. Jour. Franklin Inst. (ser. 3, v. 84) 114: 41–49.
(13) Shive, J. W.
1915. A STUDY OF PHYSIOLOGICAL BALANCE IN NUTRIENT MEDIA. Physiol. Researches 1: 327–397, illus.
(14) Swingle, D. B., and Morris, H. E.
1911. A PRELIMINARY REPORT ON THE EFFECTS OF ARSENICAL COMPOUNDS UPON APPLE TREES. Phytopathology 1: 79–93, illus.
(15) Tottingham, W. E.
1914. A QUANTITATIVE CHEMICAL AND PHYSIOLOGICAL STUDY OF NUTRIENT SOLUTIONS FOR PLANT CULTURES. Physiol. Researches 1: 133–245, illus.
(16) Wanklyn, J. A.
1901. ARSENIC. Ed. 2, 107 p. London.

STUDIES OF COMMERCIAL SAUERKRAUT WITH SPECIAL REFERENCE TO CHANGES IN THE BACTERIAL FLORA DURING FERMENTATION AT LOW TEMPERATURES[1]

By LILLIAN A. PRIEM, W. H. PETERSON, and E. B. FRED, *Departments of Agricultural Chemistry and Agricultural Bacteriology, University of Wisconsin*

INTRODUCTION

The fermentation of cabbage is a spontaneous one, and the manufacturer of sauerkraut must take the undesirable microorganisms along with the desirable ones. Because of this chance inoculation, there is a wide variation in the quality of sauerkraut. A large variety of organisms is introduced with the green plant tissue when this is placed in the vats. Many of the forms on plants can not survive under the conditions in the vat, while others find the conditions very favorable and increase to enormous numbers.

Changes in oxygen tension, hydrogen-ion concentration, and fermentable carbohydrate bring about conditions which for a few days are favorable for certain kinds of bacteria, but within a few days more these conditions may change and become unsuitable for that particular kind of microorganism. A new flora better adapted to the new conditions may come to the front and persist for a shorter or longer period of time. Although the general character of the fermentation is similar in all vats, no two are exactly the same.

The extensive research which has been made on silage shows clearly that the souring of plant tissue is the result of the combined action of many types of microorganisms. Although there are decided differences between the formation of silage and of sauerkraut, the main outlines of the two processes are the same and many of the same bacteria are found in both fermentations.

REVIEW OF LITERATURE

In the older literature on sauerkraut undue emphasis probably was placed on the activities of one type of microorganism. Conrad (*4*),[2] the first investigator in this field, isolated a culture which he regarded as the principal agent in the fermentation and to which he gave the name *Bacterium brassicae acidae*. It was a motile, Gram-negative rod which, among other products, produced hydrogen and methane. Most of the bacteria which have since been obtained from sauerkraut are nonmotile, Gram-positive, and produce neither hydrogen nor methane. *Bacterium brassicae acidae* is clearly not the usual kind of bacteria which occurs in sauerkraut.

In 1903 and 1905 Wehmer (*23*, *24*) published two papers on several aspects of sauerkraut fermentation. He concluded that gas formation was due to alcohol-forming yeasts and that acid production was caused by an organism which he tentatively named *Bacterium brassicae*. This organism was a short rod which at times appeared to be almost

[1] Received for publication Aug. 16, 1926; issued January, 1927. Published with the approval of the director of the Wisconsin Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 94.

a coccus and did not form any gas. Wehmer gives such a meager description of his organism that it is practically impossible to identify it with any culture which has since been isolated from sauerkraut. In a recent publication LeFevre (*12*) has concluded that *Bact. cucumeris fermentati*, isolated by Henneberg (*9*), and *Lactobacillus pentoaceticus*, isolated by Fred, Peterson, and Davenport (*5*), are identical with Wehmer's *Bact. brassicae*. While Wehmer says nothing of the products formed by *Bact. brassicae* other than that it formed acid, he repeatedly emphasizes the fact that *it did not form gas*. Gas production is an outstanding characteristic of *L. pentoaceticus*. Approximately 25 per cent of the glucose and other sugars is converted into carbon dioxide. Because of this and other differences it seems certain that *Bact. brassicae* and *L. pentoaceticus* are different microorganisms.

About the same time that the papers of Henneberg and Wehmer appeared, Butjagin (*3*) published a paper in which he described the successive changes in the flora of sauerkraut. He isolated and gave a good description of a short round-ended rod which in many respects resembled *Bacterium guntheri* of Gunther and Thierfelder. Aderhold (*1*) also isolated a similar organism from pickle and sauerkraut fermentations. Butjagin's bacterium differed from *Bact. guntheri* in that it did not grow well in milk and coagulated milk only after 21 to 23 days. It was Gram-positive, nonmotile, and produced no gas. In respect to the last two characteristics it was like Wehmer's *Bact. brassicae*. Wehmer makes no mention of the reaction of his organism to Gram's stain, but it is probable that it was positive. Probably both organisms are members of a large group of bacteria occurring in silage, sauerkraut, and pickles, which are strong acid producers but do not form gas. A detailed examination of the reaction of the non-gas-producing sauerkraut bacteria toward various sugars and other organic compounds shows marked differences among the various members with respect to their ability to ferment these compounds.

Shortly before Wehmer's long paper on sauerkraut appeared, Perekalin (*14*) published a short note about an acid-tolerant bacterium which he had isolated from sauerkraut. This organism was a short, nonmotile rod, negative to Gram's stain, and did not produce spores nor coagulate milk.

In 1909 Gruber (*8*) isolated an acid-producing culture which he believed to be characteristic of sauerkraut. This organism resembled Conrad's *Bacterium brassicae acidae*. It was motile, produced gas, and in other respects resembled the colon type of bacteria. He named his organism *Pseudomonas brassicae acidae*.

Round (*17*) published a short note in 1916 regarding the large numbers of bacteria in commercial suaerkraut, and concluded that bacteria alone are responsible for the fermentation. An organism which he isolated was studied by Fred, Peterson, and Davenport (*5*) in connection with the fermentation of xylose. These investigators isolated a large number of similar organisms from silage, sauerkraut, soil, manure, and cereals. They belong to the so-called "mannitol-forming" bacteria, and have received considerable attention in connection with the production of wine and butyl alcohol. In wine they produce a sour, bitter taste and in the butyl-alcohol fermentation they inhibit the growth of the organism which produces butyl alcohol.

In 1916 Henneberg (*10*) published a series of papers in which he discussed the manufacture of sauerkraut from many different angles. He divided the bacterial flora of sauerkraut into 9 or 10 groups. In the early days of the fermentation, bacteria of the colon-aerogenes type may be present, but Henneberg does not believe that they serve any useful purpose and should be excluded if possible. Oval-shaped forms, similar to *Streptococcus lactis*, are also frequently found in the early stages of the fermentation, but these gradually die off as a considerable degree of acid is attained. Henneberg attributes the odor and taste of sauerkraut chiefly to the activities of alcohol, acetic-acid-producing lactics such as *Bacterium brassicae fermentatae*. Other aroma-producing bacteria are the Pediococcus forms, of which there are many varieties. Particularly abundant in sauerkraut are the short rod forms to which group the incompletely described *Bact. brassicae* of Wehmer belongs. In old, strongly acid sauerkraut *Bact. cucumeris fermentati* and a gas-producing variety of this form are common. Other bacteria, which are less clearly defined, are chain types, flocculating types, and slime-producing types.

The functions of yeasts in the production of sauerkraut is still an unsettled question. Wehmer attached great importance to their presence, while Butjagin suggested that they might be more or less harmless, nonessential organisms. The esterlike flavors have been attributed to the compounds formed between the alcohols produced by the yeast and the acids produced by the bacteria. Peterson, Fred, and their associates (*16*, *21*) have shown that the mannitol-forming bacteria convert 25 per cent of the glucose and other sugars into ethyl alcohol. It is therefore not necessary to have yeasts in sauerkraut in order to have alcohol present.

Fred and Peterson (*6*) also showed that the so-called "pink" sauerkraut is due to yeasts. Under certain conditions the sauerkraut may become infected with large numbers of wild yeasts which produce a dirty brown color and an offensive odor and taste. On the basis of existing data no final conclusion can be drawn as to the rôle of yeasts in sauerkraut fermentation.

In 1925 Brunkow, Peterson, and Fred (*2*) published the results of a systematic study of the number and kinds of bacteria which occur in experimental sauerkraut at successive times during the fermentation period. It was desired to extend this study to commercial sauerkraut and to make a somewhat more thorough investigation of the different forms, especially as regards the occurrence of the mannitol-forming bacteria. The present paper deals with the results of this investigation.

METHODS

SOURCE OF SAMPLES

Most of the samples of sauerkraut juice were secured from a commercial sauerkraut factory, which will be designated as factory A. The samples were taken from four large vats, each vat containing about 40 tons of sauerkraut. Spigots were placed on the side near the bottom of the vats. This made it possible to draw off the juice as needed without disturbing the sauerkraut. Before a sample was taken to be analyzed some of the juice was allowed to flow through the spigot. At the beginning of the fermentation samples

were drawn at frequent intervals, but in the last part of the fermentation period samples were taken about every 30 days.

Samples of sauerkraut juice which had been in the vats from 41 days to 1 year were obtained from another factory, B.

TEMPERATURE OF VATS, ACIDITY OF SAUERKRAUT, AND NUMBER OF BACTERIA

To obtain the temperatures at which fermentation took place, a pointed metal tube was forced into the sauerkraut in such a way that the lower end was approximately at the center of the vat. Inside the tube was a thermometer, the lower part of which was inserted through a cork into a slender test tube filled with water. By this arrangement no apparent change in temperature took place for several minutes after the thermometer was withdrawn from the tube. The temperature was also taken of the sample as it was drawn from the spigot. The two temperature readings varied only a few degrees.

A 10 c. c. sample of the sauerkraut juice was diluted with an equal volume of water and, after boiling for a few seconds to expel the CO_2, was titrated with 0.1 N NaOH to the end point of phenolphthalein.

Counts of the number of bacteria were made by plating the sauerkraut juice on glucose-yeast-water agar which had been adjusted to PH 6.8. The plates were incubated at 28° C. and counted after 48 hours and again after 96 hours of incubation. After counting, 10 colonies were picked from one of the plates from each vat. As a rule the picking was made from the plate on which from 30 to 50 colonies were growing. The plate was sectioned off until there were about 10 colonies to the section. The section which appeared to have the most representative growth was selected and *all the colonies picked*. In this way 80 cultures were obtained from each of the four vats. Forty cultures were picked from the four samples of 41-day sauerkraut obtained from factory B and 10 cultures from the 1-year-old sauerkraut; in all, 370 cultures were picked.

DISCUSSION OF DATA

The temperature, acidity, and number of bacteria in the vats at various times during the fermentation are shown by the curves in Figures 1 to 4. The outstanding fact regarding these sauerkrauts is the low temperature at which fermentation took place. At the time the vats were filled the temperature was almost freezing, and at no time during the fermentation did it rise above 10.5° C. Most sauerkraut manufacturers would regard these as unusually low temperatures, but it must be noted that Wehmer considers 6° to 8° C. as the proper temperature at which to make sauerkraut. That the low temperature reduced the number of bacteria is evident from the curves in Figures 1 to 4. The highest number of bacteria in a normal fermentation is usually found from about the fifth to the seventh day. In the four vats studied the maximum was not reached until between the fifth and the twentieth day. Vat 3, which had the lowest average temperature, had an unusually low count throughout the entire fermentation, and the maximum number was not obtained until the twentieth day.

After the maximum was reached the count for all the vats rapidly dropped to a small number and continued at this low level for several weeks. After 90 days the temperature of the vats rose slightly, and

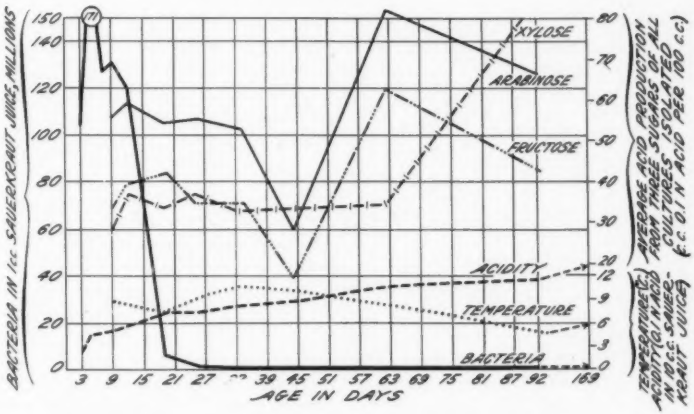

BACTERIA IN 1cc SAUERKRAUT JUICE, MILLIONS
AVERAGE ACID PRODUCTION FROM THREE SUGARS BY ALL CULTURES ISOLATED IN 10cc SAUERKRAUT JUICE (cc 0.1 N ACID PER 100 cc)
TEMPERATURE (°C.) ACIDITY (cc 0.1 N ACID IN 10cc SAUERKRAUT JUICE)
XYLOSE
ARABINOSE
FRUCTOSE
ACIDITY
TEMPERATURE
BACTERIA
AGE IN DAYS

FIG. 1.—Temperature, acidity, and number of bacteria at various times during the formation of sauerkraut; also the acidity produced from certain sugars by sauerkraut bacteria. (Vat 1)

this rise was accompanied by a small increase in the number of bacteria.

In the first days of the fermentation numerous peptolytic catalase-positive bacteria were present, but these soon disappeared. Coccus

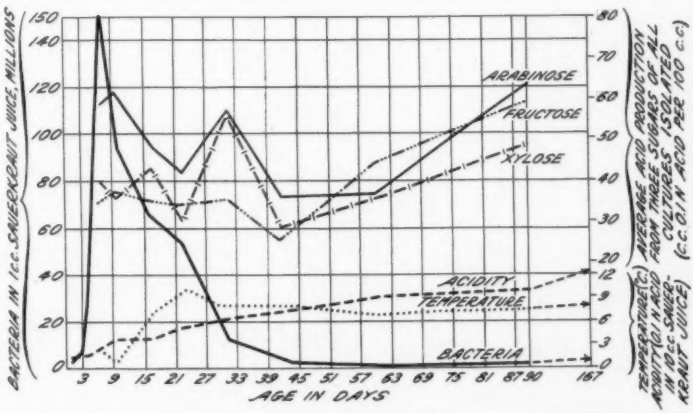

BACTERIA IN 1cc SAUERKRAUT JUICE, MILLIONS
AVERAGE ACID PRODUCTION FROM THREE SUGARS BY ALL CULTURES ISOLATED IN 10cc SAUERKRAUT JUICE (cc 0.1 N ACID PER 100 cc)
TEMPERATURE (°C.) ACIDITY (cc 0.1 N ACID IN 10cc SAUERKRAUT JUICE)
ARABINOSE
FRUCTOSE
XYLOSE
ACIDITY
TEMPERATURE
BACTERIA
AGE IN DAYS

FIG. 2.—Temperature, acidity, and number of bacteria at various times during the formation of sauerkraut; also the acidity produced from certain sugars by sauerkraut bacteria. (Vat 2)

forms predominated in the early stages of the fermentation; short rods were present all the time, but were more abundant during the latter part, and long rods appeared only toward the end of the fermentation. All four vats showed a slow increase in acid, but none

reached a particularly high amount. At the end of 90 days about 1 per cent of acid, calculated as lactic, was found. Samples of the sauerkraut juice were then analyzed for reducing sugars, and the

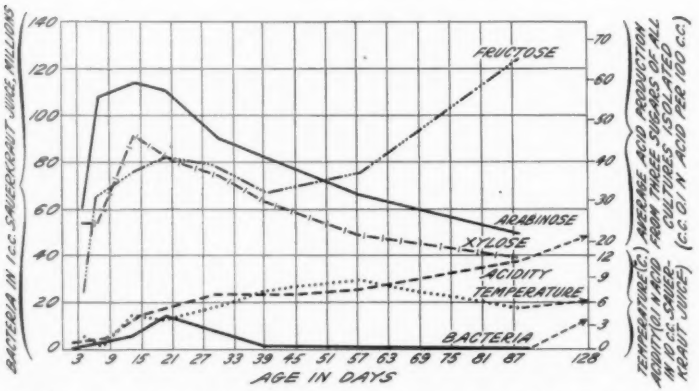


FIG. 3.—Temperature, acidity, and number of bacteria at various times during the formation of sauerkraut; also the acidity produced from certain sugars by sauerkraut bacteria. (Vat 3)

surprising fact was revealed that there was practically none left. Apparently complete fermentation had taken place at the low temperatures of the vats, but only a small part of the sugar had been converted into acids. A complete analysis of the sauerkraut juice

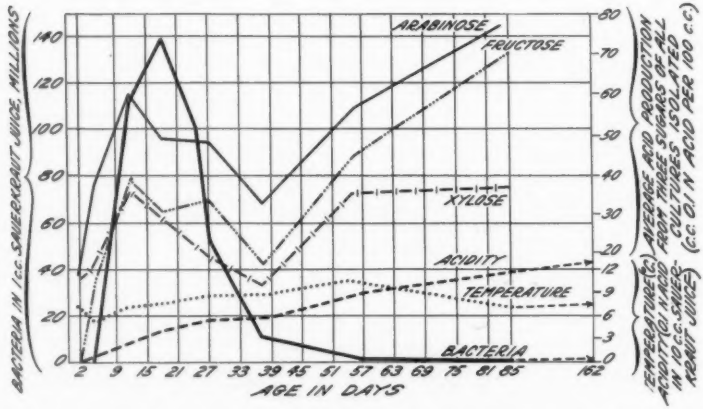


FIG. 4.—Temperature, acidity, and number of bacteria at various times during the formation of sauerkraut; also the acidity produced from certain sugars by sauerkraut bacteria. (Vat 5)

was therefore made to determine, if possible, what other fermentation products had been formed. The methods used have been described in previous publications (*18*, *19*, *5*, *15*).

TABLE 1.—*Fermentation products contained in sauerkraut juice from different vats (factory A)*

No. of vat	Temperature [a]	Age of sauerkraut	Sugar	Titratable acid as lactic	Volatile acid as acetic	Nonvolatile acid as lactic	Alcohol as ethyl
	° C.	*Days*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Vat 1	8.0	92	0.12	1.026	0.270	0.663	0.432
Vat 2	6.9	90	.10	.873	.266	.575	.653
Vat 3	5.3	87	.12	1.017	.276	.703	.920
Vat 5	7.7	85	.10	.972	.334	.678	.680

[a] Average for entire fermentation period, from 128 to 169 days.

FERMENTATION PRODUCTS

The percentage of volatile acid, nonvolatile acid, alcohol, and unfermented sugar contained in the sauerkrauts are given in Table 1. The percentage of acetic acid is normal but the amount of lactic acid is low. In a normal fermentation there is from three to five times as much lactic acid as acetic acid, but in the sauerkrauts studied the ratio is only from 2 to 2.5. The alcohol content ranged from 0.43 to 0.92 per cent, with an average of 0.67 per cent. The average for nine commercial sauerkrauts was found by Fred and Peterson (7) to be 0.47 per cent. The analytical data indicate that the low temperatures of these vats favored the development of alcohol-producing bacteria. It is possible that, if mannitol could have been determined on these samples, an unusually high percentage would have been found. No satisfactory determination could be obtained because of the interference of salt. When the vats were opened the sauerkraut was graded and was rated fair. From this limited evidence it would seem that low temperatures do not give the best quality of sauerkraut. More data must be obtained, however, before a final conclusion on this point can be drawn.

KINDS OF BACTERIA IN THE SAUERKRAUTS

A rough classification of the several hundred bacteria picked from the plates was made by inoculating 10 c. c. of 1 per cent yeast-water solutions of fructose, arabinose, and xylose with these cultures and determining the acidity formed after seven days of incubation. The titrations for the 10 cultures picked at each time of sampling were averaged for each sugar and the results plotted in the form of curves as shown in Figures 1 to 4.

While there are many irregularities in the curves, the acid production from these sugars seems to follow the bacterial curve during the first half of the fermentation. When the number of bacteria decreased, the acid-producing ability of the bacteria also decreased. The decline in acid production was not so great, however, as in numbers of bacteria, and after six weeks a general increase in acid production took place. A high acid-producing type of bacteria came to the front. These were probably the long rods which are usually abundant in old sauerkraut.

The cultures were also inoculated into litmus milk, but less than 50 per cent produced any reduction or curd in this medium and only a few digested milk. Those which did produce proteolytic changes in litmus milk were cultures which had been obtained in the early

stages of the fermentation. These bacteria were undoubtedly plant forms which did not find the conditions of the vat suitable for their existence.

The cultures were also tested for catalase with hydrogen peroxide, but more than 80 per cent were found negative. The great majority of bacteria in sauerkraut are catalase-negative, produce little or no change in milk, but ferment arabinose, xylose, and fructose readily with the production of considerable quantities of acid. The large number of pentose fermenters corroborates the findings reported in a previous paper (*2*) in which it was stated that the xylose fermenters practically equalled the number that attacked glucose.

FERMENTATION CHARACTERISTICS OF THE SELECTED CULTURES

On the basis of the above five tests 90 of the 320 cultures were selected for further study. In making this selection an effort was made to include representatives of all the various types encountered. Tables 2 to 5 give the representative types found in the four commercial vats at factory A. Table 6 contains the data for the selected cultures obtained from factory B. Numbers 60–2, 60–6, and 60–11 were selected from the year-old sauerkraut and all others in this table came from the 41-day sauerkraut. A few of the organisms in Table 6 are outstanding because of the great acidity developed in the sugars. Organisms in Tables 2 to 5, selected from factory A, where a low temperature was maintained, do not show such a marked ability to produce acid. Most of the cultures slowly produce acid in litmus milk and about half of them bring about some other change such as curdling, reduction, or granule formation. The mannitol formers produce less acidity and softer curds than the nonmannitol bacteria. Reduction of litmus is also more prominent with the latter group.

TABLE 2.—*Fermentation characteristics of the selected cultures from vat 1 (factory A)*

Culture No.	Litmus milk after 30 days	Gas from glucose	0.1 N acid in 100 c. c. of culture			
			Arabinose	Xylose	Glucose	Fructose
			C. c.	*C. c.*	*C. c.*	*C. c.*
40–1	No change	*+	72	54	78.9	50.0
40–3	do	+	62	22	75.8	47.2
40–6	do	+	76	50	73.1	48.4
41–2	do	+	72	50	75.9	71.6
41–10	do	+	78	48	75.9	46.8
43–8	do	+	78	50	84.8	52.0
44–1	do	+	88	62	75.9	53.6
45–3	do	+	69	50	86.0	50.0
46–2	do	+	51	115	96.0	58.0
46–8	Reduced	+	98	114	90.0	51.2
46–10	do	+	102	109	100.0	55.2
47–9	No change	+	106	120	94.0	50.4
48–1	do	+	36	112	80.0	49.0
48–3	do	+	134	107	82.0	50.0
48–6	Reduced	−	79	28	96.0	97.0

* Plus sign indicates presence of gas; minus sign, absence of gas.

TABLE 3.—*Fermentation characteristics of the selected cultures from vat 2 (factory A)*

Culture No.	Litmus milk after 30 days	Gas from glucose	0.1 N acid in 100 c. c. of culture			
			Arabinose	Xylose	Glucose	Fructose
			C. c.	*C. c.*	*C. c.*	*C. c.*
50–2	No change	+	82	52	81.8	52.0
50–5	do	+	72	66	85.0	50.4
51–9	Soft curd	+	86	50	82.0	53.2
53–4	No change	+	24	68	81.8	52.8
53–5	do	+	70	58	58.4	51.6
53–8	do	+	22	78	80.5	52.0
54–1	do	−	18	26	54.0	54.0
54–5	do	+	74	56	82.4	56.4
54–10	Granules	−	74	24	62.4	73.0
55–4	No change	+	90	52	84.0	52.0
55–6	do	+	32	76	82.0	51.6
55–9	do	+	80	80	88.0	54.4
56–2	Reduced	+	76	59	102.0	52.4
56–7	No change	−	15	16	86.0	32.8
57–2	Reduced	−	19	21	98.0	69.2
57–3	do	−	120	28	90.0	60.8
57–7	do	+	115	114	86.0	45.2
57–8	No change	+	67	60	88.0	47.2
58–3	do	−	22	18	96.0	82.0
58–6	do	+	120	128	102.0	78.0
58–8	Reduced	−	106	32	104.0	104.0

TABLE 4.—*Fermentation characteristics of the selected cultures from vat 3 (factory A)*

Culture No.	Litmus milk after 30 days	Gas from glucose	0.1 N acid in 100 c. c. of culture			
			Arabinose	Xylose	Glucose	Fructose
			C. c.	*C. c.*	*C. c.*	*C. c.*
21–1	No change	+	64	54	76.0	50.4
21–2	Reduced, curd	+	36	22	54.0	4.8
22–3	No change	+	82	26	77.4	48.4
22–5	do	+	94	54	90.5	47.6
22–8	do	+	72	46	62.8	93.2
22–10	Peptonized	+	34	40	53.1	8.0
24–1	No change	+	80	74	74.4	54.4
25–6	do	+	86	60	80.5	55.6
25–7	Reduced, curd	−	76	70	65.6	71.6
26–4	Reduced	+	62	46	84.0	62.4
26–8	No change	+	64	59	57.2	90.0
27–1	Reduced	+	63	44	78.0	49.2
27–8	do	+	78	62	84.0	45.2
28–5	No change	−	59	19	64.0	60.4
28–7	Reduced	+	66	104	80.0	58.4
28–8	No change	−	19	25	88.0	67.6
29–1	Reduced	−	66	38	94.0	99.0
29–4	No change	+	127	115	104.0	73.0
29–6	do	−	21	19	104.0	97.0
29–7	do	−	28	22	82.0	99.0

TABLE 5.—*Fermentation characteristics of the selected cultures from vat 5 (factory A)*

Culture No.	Litmus milk after 30 days	Gas from glucose	0.1 N acid in 100 c. c. of culture			
			Arabinose	Xylose	Glucose	Fructose
			C. c.	*C. c.*	*C. c.*	*C. c.*
30-2	Reduced, curd	+	42	30	39.4	3.6
30-7	Reduced	+	27	48	50.6	11.6
31-1	No change	+	94	24	74.5	48.0
31-2	Reduced, curd	−	10	28	55.0	12.0
31-8	do	+	40	38	40.8	8.4
32-2	do	−	70	70	73.6	57.6
32-6	No change	+	88	50	73.1	55.2
33-1	do	+	72	50	50.0	70.1
33-2	do	+	72	44	71.6	50.8
34-2	Reduced	+	80	42	90.0	51.2
34-4	do	+	53	39	88.0	51.2
34-7	do	+	66	76	84.0	47.6
35-1	No change	−	65	17	74.0	22.8
35-7	Reduced	+	78	52	90.0	48.8
35-10	do	+	61	42	86.0	50.8
36-2	Reduced, curd	−	81	35	100.0	95.6
36-4	Reduced	+	108	133	100.0	51.6
36-6	Reduced, curd	−	23	34	108.0	88.0
37-2	Reduced	−	109	28	104.0	94.0
37-3	do	+	70	48	92.0	91.0
37-6		+	102	125	88.0	75.0

TABLE 6.—*Fermentation characteristics of the selected cultures from factory B*

Culture No.	Litmus milk after 30 days	Gas from glucose	0.1 N acid in 100 c. c. of culture			
			Arabinose	Xylose	Glucose	Fructose
			C. c.	*C. c.*	*C. c.*	*C. c.*
60-2	Reduced	+	120	116	86	106
60-6	do	+	84	105	98	54
60-11	Reduced, curd	−	27	25	106	100
62-3	do	−	20	23	90	96
62-6	No change	+	120	109	126	76
62-10	Reduced, curd	−	73	39	78	101
63-5	Reduced	+	119	109	96	71
64-2	Reduced, curd	−	69	30	100	91
64-7	Reduced	+	118	22	84	118
64-8	do	−	20	24	88	154
64-9	Reduced, curd	+	114	106	92	87
65-5	do	−	66	34	98	201
65-9	Reduced	−	19	22	90	152

GAS FORMATION

The cultures were inoculated into 10 c. c. of 1 per cent glucose-yeast water, sealed with sterile melted vaseline and allowed to ferment at 28° C. They were observed from day to day for gas formation. If the vaseline plug was pushed up they were recorded as gas-positive. If the vaseline remained at the surface of the liquid and there was no apparent leakage of gas, they were called negative. At the end of seven days the vaseline plug was removed and the culture titrated for acidity with 0.1 N. NaOH. If acid had developed it was evident that the organisms had grown and the negative results were not due to a failure to grow.

Of the 90 cultures, 63 formed gas and 27 did not. The proportion of gas-forming organisms was higher in the vats from factory A than

from factory B. In the first factory there were almost three times as many gas formers as nongas formers, while in the second factory the numbers were about equal. Low temperatures appear to favor the development of gas-producing bacteria. As will be shown later, gas and mannitol formation coincide in most cases. Certain cultures, however, produced no gas but formed a small quantity of mannitol.

Reaction to Gram's Stain

In most cases the gas-producing organisms were also positive to Gram's stain. Most of the organisms were short rods, but some of those picked from the young sauerkraut were cocci. In the later stages of the fermentation a considerable number of long rods appeared.

RESELECTED AND PURIFIED CULTURES

On the basis of the tests just described the 90 cultures were gone over and 18 were chosen as representative of all the types found in the larger group. These were replated and new colonies picked. The purified cultures were inoculated into 10 c. c. of 1 per cent yeast-water solution of melezitose, raffinose, and salicin, and after seven days' incubation titrated for acid. Their ability to form mannitol from fructose was also determined by fermenting 75 c. c. of 2 per cent fructose-yeast water containing an excess of $CaCO_3$. After seven days the fermented medium was carefully decanted from the excess $CaCO_3$ and evaporated to 5 to 10 c. c. on the steam bath and then allowed to go to dryness at room temperature. If the culture is a good mannitol former, crystals of mannitol will appear throughout the residue and frequently extend up the sides of the beaker. The test is usually very definite, but if doubt existed the fermentation was repeated. The cultures in which no crystals appeared the second time were acidified with H_2SO_4 and extracted four times with ether to remove lactic acid, which tends to hide the mannitol crystals. The residue from the ether extraction was then extracted six times with hot 80 per cent alcohol and the extract concentrated to a small volume on the steam bath and then evaporated spontaneously at room temperature. If no mannitol crystals appeared the cultures were classed as nonmannitol-forming. The results of this test and the other characteristics of the 18 cultures are summarized in Table 7. This list includes cultures which ferment both pentoses, some of which ferment arabinose but not xylose, and some which ferment neither. Only 3 cultures were obtained which fermented xylose but not arabinose. Such a microorganism has been reported in a previous publication (*21*), but the arabinose-negative, xylose-positive type is much less common than the reverse combination.

Only four cultures were found to ferment melezitose, and all of these were mannitol-formers. The converse is not true, however; several cultures formed mannitol but did not ferment melezitose.

If 25 c. c. of 0.1 N acid be taken as indicating fermentation, more than half of the 18 cultures attack raffinose and salicin. According to Orla-Jensen (*13*), the ability to ferment salicin is rare among the lactic-acid bacteria of green vegetable matter.

TABLE 7.—*Fermentation characteristics of selected cultures*

Culture No.	Gas	Litmus milk	Mannitol formation	0.1 N acid in 100 c. c. of culture						
				Arabinose	Xylose	Glucose	Fructose	Melezitose	Raffinose	Salicin
				C. c.	*C. c.*	*C. c.*	*C. c.*	*C. c.*	*C. c.*	*C. c.*
29–7	−	−	−	28	21	82	99	16	16	56
36–6	−	+	+	23	34	108	88	75	89	74
54–10	−	−	−	74	24	63	62	24	33	25
25–7	−	+	−	76	70	67	72	17	19	33
32–2	+	+	+	70	70	73	58	17	58	55
31–2	−	+	−	10	28	55	12	8	18	29
64–2	−	+	+	69	30	100	91	67	85	80
57–8	+	−	+++	67	60	88	47	17	25	18
58–8	−	+	+	106	32	104	104	84	63	88
22–10	+	+	−	34	40	53	8	23	40	27
46–2	+	−	+++	51	115	96	58	19	18	20
51–9	+	+	+	86	50	80	53	15	43	80
22–8	+	−	++	72	46	63	93	17	48	57
55–9	+	+	++	80	80	88	54	20	42	48
48–3	+	−	+++	134	107	82	50	28	22	23
36–4	+	+	+++	108	133	100	51	19	20	21
64–9	+	+	++	114	106	92	87	19	62	23
26–8	+	−	++	64	59	57	90	17	38	52

A large majority of the organisms are mannitol formers but vary in the quantity which they produce. Those forming a small quantity are indicated with one plus sign in the table, while those forming a large quantity are indicated with two or three plus signs. Three of the mannitol formers, Nos. 36–6, 64–2, and 58–8, were conspicuous because of the large deposit of calcium salts which separated out with the mannitol crystals. They also failed to form gas from either glucose or fructose. The tests for gas and mannitol formation were repeated twice to make sure there was no error. The quantity of mannitol was small but unmistakable. Apparently the formation of mannitol is a property which varies from traces to 50 to 60 per cent of the fructose consumed. In 1921 Kayser (*11*) reported a similar case of mannitol formation by lactic-acid bacteria which did not form any gas.

Several of the cultures are noteworthy in that they produce no turbidity in the media. Floccules appear on the sides of the tube or settle to the bottom, leaving the medium perfectly clear. Cultures 25–7 and 64–9 were conspicuous in this respect. Considerable importance has been attached to the property of floccule formation in a recent classification of lactic-acid bacteria by Van Steenberge (*20*)

QUANTITATIVE DETERMINATION OF THE FERMENTATION PRODUCTS FROM ARABINOSE, FRUCTOSE, AND GLUCOSE

Six of the mannitol formers were selected for quantitative work. Differences in the fermentation of the sugars formed the basis of the selection. From the following summary it can be seen at a glance what the differences are. The minus sign signifies that there was little or no fermentation; the plus sign indicates good fermentation, and the double plus very good fermentation.

Culture No.	Arabinose	Xylose	Glucose	Fructose	Melezitose	Raffinose	Salicin
36–6	−	−	+	+	+	+	+
64–2	+	−	+	+	+	+	+
57–8	+	+	+	+	−	−	−
36–4	++	++	+	+	−	−	−
55–9	+	+	+	+	−	+	+
64–9	+	+	+	+	−	+	−

The cultures were inoculated into 300 c. c. of sugar-yeast-water media containing about 2 per cent of the sugar. The fermentation flask was equipped with a trap and absorption bottle for collecting the CO_2. Sterile bromo-cresol purple was added to the flask, and as fermentation proceeded the acid which formed was neutralized with sterilized 1 N NaOH. When acid production ceased the culture was acidified with H_2SO_4 and aspirated for 30 minutes to remove and absorb any traces of CO_2 in the flask. The KOH solution was made to a definite volume and analyzed for CO_2 by the Van Slyke (*22*) apparatus. The fermented medium was made to 400 c. c. and aliquots analyzed for unfermented sugar, alcohol, volatile acid, nonvolatile acid, and mannitol by methods already described (*18*, *19*, *5*, *15*). The data are given in Table 8.

TABLE 8.—*Fermentation products of arabinose, glucose, and fructose*

[Calculated for 100 c. c. of culture]

Culture No.	Age	Sugar		Acids		Alcohol as ethyl	Carbon dioxide	Mannitol	Total products
		Kind	Amount fermented	Volatile as acetic	Nonvolatile as lactic				
	Days		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
57–8	7	Arabinose	1.823	0.841	0.937	0.012	0.040	----------	1.830
36–4	7	do	1.829	.806	.983	.005	.036	----------	1.830
55–9	12	do	1.138	.427	.674	.006	.026	----------	1.133
58–8	11	do	1.938	.347	1.412	.008	.047	----------	1.814
64–9	8	do	1.864	.379	1.485	.005	.041	----------	1.910
64–2	8	do	1.938	.505	1.319	.008	.062	----------	1.894
57–8	36	Glucose	1.225	.085	.438	.248	.270	----------	1.041
64–9	34	do	1.700	.133	.620	.297	.365	----------	1.415
36–4	15	do	.747	.052	.281	.141	.116	----------	.590
55–9	15	do	1.497	.047	.658	.319	.314	----------	1.338
64–2	34	do	1.110	.051	.846	.008	.042	----------	.947
36–6	36	do	1.940	.066	1.728	.019	.046	----------	1.859
57–8	12	Fructose	1.550	.288	.377	.055	.223	0.516	1.459
64–9	14	do	1.615	.257	.677	.129	.344	.232	1.639
36–4	12	do	1.580	.299	.580	.082	.246	.173	1.380
55–9	8	do	1.068	.234	.438	.051	.159	.103	.985
36–6	17	do	1.550	.044	1.429	.029	.066	?	1.568
64–2	14	do	1.615	.052	1.558	.013	.048	?	1.671

FERMENTATION OF ARABINOSE

This sugar was fermented very vigorously by all the cultures with the exception of No. 36–6 which does not attack this pentose. The principal products formed were volatile and nonvolatile acids. Only traces of CO_2 and alcohol were formed.

There was great variation in the quantity of products formed. The volatile acid comprised from 19 to 47 per cent and the nonvolatile acid from 50 to 78 per cent. On the basis of arabinose fermented, the lactic acid amounts to more than 70 per cent of the sugar. If more than 60 per cent of the sugar is converted into lactic acid, it is necessary to assume that parts of two molecules of arabinose are drawn upon to produce the extra 10 or more per cent of lactic acid. Only one 3-carbon compound can be obtained per molecule of pentose unless the remaining 2-carbon portions are first combined and then broken down to yield a 3-carbon compound such as lactic acid. A synthesis as well as a cleavage must therefore be brought about by the

bacteria. Cultures 58–8, 64–2, and 64–9 are all outstanding because of their ability to convert more than 60 per cent of the arabinose into lactic acid.

Fermentation of Glucose

The six cultures fall into two groups. Nos. 36–6 and 64–2 are straight lactics, while the other four form considerable quantities of alcohol and carbon dioxide with a small amount of volatile acid. These four cultures break up the glucose approximately as follows: $C_6H_{12}O_6 = C_3H_6O_3 + C_2H_5OH + CO_2$.

The actual figures show a slight departure from the theoretical, but in most cases this is less than 5 per cent.

Fermentation of Fructose

Fructose was rapidly and almost completely destroyed. Large quantities of lactic acid were formed by Nos. 36–6 and 64–2, but no mannitol could be found. Whether mannitol is formed under the somewhat acid conditions of the fermentation or whether its presence could not be detected because of interfering substances is not known. Mannitol was formed in the presence of $CaCO_3$, but the amount was too small to determine quantitatively. The remaining four cultures were very similar in the products formed. The volatile acids varied from 16 to 24 per cent.

Mannitol, carbon dioxide, and acetic acid production run parallel to one another and inversely to the production of lactic acid. The cultures which produced much mannitol also produced large quantities of CO_2. Appreciable quantities of ethyl alcohol were formed only by such cultures as produced considerable quantities of mannitol.

Form of Lactic Acid Produced

The form of lactic acid is readily identified by the water of crystallization of its zinc salt. Table 9 gives the data for these salts. Most of the lactic acid produced by these organisms was inactive. Culture 55–9 differs from the other cultures in producing an excess of one form of lactic acid. The water of crystallization is intermediate between that for inactive and active zinc lactate. To determine which enantiomorph was in excess, the zinc salts were examined in a polariscope. The first crop from arabinose and fructose was tested in each case and both were found dextrorotatory. Since the rotation of the zinc salt is opposite to that of the free acid, evidently culture 55–9 produces more *l*- than *d*-lactic acid.

TABLE 9.—*Forms of lactic acid produced by various cultures*

Culture No.	Sugar	Zinc lactate			Extracted acid recovered as zinc salt
		Crop No.	Weight	Water of crystallization [a]	
			Gms.	*Per cent*	*Per cent*
64-2	Glucose	1	0.8173	18.1	65
64-9	do	1	.5711	18.1	60
36-6	do	1	2.1144	18.1	81
57-8	do	1	.4116	18.0	59
64-2	Fructose	1	2.0902	18.1	81
64-9	do	1	.6636	17.7	54
		2	.1284	17.5	
36-4	do	1	.5875	18.2	52
		2	.0666	18.1	
36-6	do	1	2.2923	18.2	78
55-9	do	1	.1022	15.4	40
		2	.1913	13.8	
57-8	do	1	.4626	17.8	51
		2	.0436	17.9	
64-2	Arabinose	1	1.5252	18.4	61
		2	.1281	17.8	
64-9	do	1	1.7636	18.2	96
36-4	do	1	1.1992	18.5	88
		2	.1631	17.2	
55-9	do	1	.7467	14.3	68
57-8	do	1	1.0091	18.5	92
		2	.3527	18.5	

[a] Theory for H_2O in $Zn(C_3H_5O_3)_2+3H_2O=18.2$; theory for H_2O in $Zn(C_3H_2O_3)_2+2H_2O=12.9$.

SUMMARY

The acidity, temperature, and number of bacteria in four vats of commercial sauerkraut were determined at different intervals for a period of more than five months. There was a slow but regular increase in acid which, calculated as lactic, was equivalent to about 1 per cent after three months, and about 1.3 per cent after five months. The temperature in all of these vats was unusually low. At no time did it rise above 10.5° C., and this fact was undoubtedly responsible for the slow development of acid. There was a rapid increase in the number of bacteria from the second to the twentieth day, after which there was a big drop in the number of microorganisms.

Pure cultures were picked from each plating until a total of 370 were obtained. Most of these organisms fermented arabinose, xylose, and fructose but produced little change in litmus milk. Practically all were catalase-negative.

The fermentation characteristics of representative cultures of the 370 were studied further. A few of these were coccus forms but most of them were rods. The large majority were Gram-positive and catalase-negative. About two-thirds formed gas from glucose and approximately the same number formed mannitol from fructose. Several new strains of mannitol-forming bacteria were found.

Six of the mannitol-forming cultures were used for the quantitative determination of their fermentation products. Lactic acid was the principal product, and varied from 36 per cent in the case of glucose to 79 per cent in the case of arabinose. Such a large percentage of lactic acid can not be obtained by direct cleavage of the pentose molecule, but must involve synthesis of portions from two or more molecules. In most cases approximately equal quantities of *d*- and

l-lactic acid were produced. One culture, No. 55–9, produced an excess of the *l* form.

Besides lactic acid, acetic acid, and ethyl alcohol, carbon dioxide, and mannitol were produced in quantities which varied with the sugar and the culture used.

LITERATURE CITED

(1) ADERHOLD, R.
1906. SAUERKRAUT, KOMST, STSCHI. *In* Lafar, F., Handbuch der technischen Mykologie, Aufl. 2, Bd. 2, p. 319–322. Jena.

(2) BRUNKOW, O. R., PETERSON, W. H., and FRED, E. B.
1925. A STUDY OF THE INFLUENCE OF INOCULATION UPON THE FERMENTATION OF SAUERKRAUT. Jour. Agr. Research 30: 955–960.

(3) BUTJAGIN, B.
1904. VORLÄUFIGE MITTEILUNG ÜBER SAUERKRAUTGÄRUNG. Centbl. Bakt. [etc.] (II) 11: 540–550.

(4) CONRAD, E.
1897. BACTERIOLOGISCHE UND CHEMISCHE STUDIEN ÜBER SAUERKRAUTGÄHRUNG. Arch. Hyg. 29: 56–95, illus.

(5) FRED, E. B., PETERSON, W. H., and DAVENPORT, A.
1919. ACID FERMENTATION OF XYLOSE. Jour. Biol. Chem. 39: 347–384, illus.

(6) ——— and PETERSON, W. H.
1922. THE PRODUCTION OF PINK SAUERKRAUT BY YEASTS. Jour. Bact. 7: 257–269.

(7) ——— and PETERSON, W. H.
1924. FACTORS DETERMINING QUALITY IN KRAUT. Canning Age 1924: 161–165, illus.

(8) GRUBER, T.
1909. UEBER SAUERKRAUTGÄRUNG UND IHRE ERREGER. Centbl. Bakt. [etc.] (II) 22: 555–559.

(9) HENNEBERG, W.
1903. ZUR KENNTNIS DER MILCHSÄUREBAKTERIEN DER BRENNEREIMAISCHE, DER MILCH, DES BIERES, DER PRESSHEFE, DER MELASSE, DES SAUERKOHLS, DER SAUREN GURKEN, UND DES SAUERTEIGS, SOWIE EINIGE BEMERKUNGEN ÜBER DIE MILCHSÄUREBAKTERIEN DES MENSCHLICHEN MAGENS. Centbl. Bakt. [etc.] (II) 11: 154–170.

(10) ———
1916. DAS SAUERKRAUT (SAUERKOHL). Deut. Essigindus. 20: 133–136, 141–144, 152–155, 160–161, 166–170, 176–177, 184–185, 192–194, 199–202, 207–209, 215–216, 223–225, illus.

(11) KAYSER, E.
1921. LA FERMENTATION LACTIQUE. Lait 1: 184–191.

(12) LEFEVRE, E.
1922. PICKLE AND SAUERKRAUT EXPERIMENTS. (Abstract) Abs. Bact. 6: 24–25.

(13) ORLA-JENSEN, S.
1919. THE LACTIC ACID BACTERIA. K. Danske Vidensk. Selsk. Skr., Naturv. og Math. Afd. (8) 5: 81–196, illus.

(14) PEREKALIN.
1905. [UEBER EIN AUS SAUERKOHL AUSGESCHIEDENES ACIDOPHILES BAKTERIUM.] Centbl. Bakt. [etc.] (II) 14: 225–226.

(15) PETERSON, W. H., and FRED, E. B.
1920. FERMENTATION OF FRUCTOSE BY LACTOBACILLUS PENTOACETICUS, N. SP. Jour. Biol. Chem. 41: 431–450, illus.

(16) ——— and FRED, E. B.
1920. THE FERMENTATION OF GLUCOSE, GALACTOSE, AND MANNOSE BY LACTOBACILLUS PENTOACETICUS N. SP. Jour. Biol. Chem. 42: 273–287.

(17) ROUND, L. A.
1916. NORMAL FERMENTATION OF SAUERKRAUT. (Abstract) Jour. Bact. 1: 108.

(18) Shaffer, P. A., and Hartmann, A. F.
1921. the iodometric determination of copper and its use in sugar analysis. I. equilibria in the reaction between copper sulfate and potassium iodide. Jour. Biol. Chem. 45: 349–364, illus.
(19) ——— and Hartmann, A. F.
1921. the iodometric determination of copper and its use in sugar analysis. II. methods for the determination of reducing sugars in blood, urine, milk, and other solutions. Jour. Biol. Chem. 45: 365–390, illus.
(20) Steenberge, P. van
1920. les propriétés des microbes lactiques; leur classification. Ann. Inst. Pasteur 34: 803–870, illus.
(21) Stiles, H. R., Peterson, W. H., and Fred, E. B.
1925. fermentation products of certain mannitol-forming bacteria. Jour. Biol. Chem. 64: 643–654.
(22) Van Slyke, D. D.
1917. studies of acidosis. II. a method for the determination of carbon dioxide and carbonates in solution. Jour. Biol. Chem. 30: 347–368, illus.
(23) Wehmer, C.
1903. die sauerkrautgärung. Centbl. Bakt. [etc.] (II) 10: 625–629.
(24) ———
1905. untersuchungen über sauerkrautgärung. Centbl. Bakt. [etc.] (II) 14: 682–713, 781–800, illus.